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THE
Journal of Parasitology

A QUARTERLY DEVOTED TO MEDICAL ZOOLOGY

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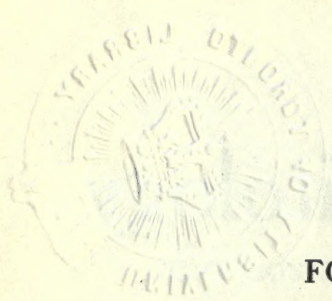
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The Journal of Parasitology

Volume 4

SEPTEMBER, 1917

Number 1

ON THE STRUCTURE AND CLASSIFICATION OF NORTH AMERICAN PARASITIC WORMS *

HENRY B. WARD

For many years I have been engaged in the study of parasitic worms from North American freshwater hosts, mostly fish; during this time I have had opportunity to examine and compare material from a large number of localities embracing many widely separated points. In this work I have been aided very greatly by studies on individual groups undertaken and published under my direction by various graduate students to whom my obligation is freely expressed here. In connection with this work it has been necessary to examine critically all original records of parasites from similar hosts and to endeavor to reach a positive determination of the parasite under discussion in each case. This is not a simple matter, as the records were often made on the basis of a rapid preliminary examination; furthermore, the total lack of special reference works on these groups led to the recording of parasitic species under general names taken from the older European writers, and these names are often wanting in definite significance.

My work has naturally led to the discovery of new facts regarding the structure of the forms studied and has compelled me to introduce new names and to rearrange forms so as to express better their correct relationships in the light of more perfect knowledge of their structure. Such changes are of course unfortunate in that they make it difficult to trace the continuity between the new and the old in zoological literature; they are nonetheless essential if the student is to apprehend the true character and affiliations of the forms with which he comes in contact. It has been my fixed principle never to make any changes until I was personally familiar with the form discussed or had acquired such acquaintance with its structure as to know that some change was inevitable and that the proposed modification was defensible on morphological grounds. Most of the questions involved in the changes listed later in this paper have been submitted to the criticism of advanced workers in the field, or discussed before graduate classes for some years so that they may be regarded as seasoned changes.

* Contributions from the Zoological Laboratory of the University of Illinois, No. 94.

Some of the more general results of my work have been included in brief form in synopses of the Parasitic Flatworms and Parasitic Roundworms which constitute two chapters in *Freshwater Biology* by Ward and Whipple just being published by John Wiley and Sons, New York. It seems to me wise to print here in outline the most important new material regarding the structure and classification of the parasitic forms discussed in these chapters as there are items which might easily escape notice and thus lead to confusion if published only in a textbook. The work just cited gives complete summaries of the North American forms in the groups mentioned and the place of the items discussed later in this paper may be precisely determined by reference to it. These items are arranged here in the order in which they are taken up in the book, and this is the systematic arrangement. The student will also find there an abundance of illustrations to demonstrate the points discussed here.

TAXONOMIC CHANGES AMONG TREMATODA

The genus *Polystoma* established by Zeder in 1800 is well known thru the common European type, *P. integerrimum*, generally employed in text books to illustrate the group of monogenetic trematodes. Several species from North America have been carefully described by Stunkard (1917). These forms stand out distinctly when compared with the European type and clearly constitute a separate section of the genus to which I have given the name *Polystomoides*. This subgenus which further study may show to be of generic rank is characterized by the presence of a short uterus containing only a single egg whereas the European type possessed a uterus of several coils with numerous eggs. Stunkard has also pointed out considerable differences in the structure of the suckers on the caudal disc. As type may be designated *P. (Polystomoides) coronatum* (Leidy 1888) from the common food terrapin.

As the cause of an epidemic among sparrows at Madison, Wisconsin, Cole (1911) reported under the name of *Monostoma faba* a trematode that in reality differs distinctly from the European species. The form of the ovary, the extent of the vitellaria, the dermal spines, and other details of structure disagree with the recent description of Kossack who moreover assigned Rudolphi's species to his new genus *Collyriclum*. The American form constitutes a new species in this genus and to it the name *Collyriclum colei* may be given. The position of this genus is so isolated among the monostomes that a new family must be created for it. This may be characterized as follows:

COLLYRICLIDAE Ward. Small to moderate sized monostomes with discoidal, compressed, not muscular body, broader than long. Oral sucker weak; pharynx present; ceca simple, long, capacious, not united. Genital pore ventral, near center of body. Vitellaria follicular, scanty, antero-lateral; ovary much lobed, asymmetrical. Uterus posterior, in irregular coils which show an antero-posterior tendency, terminal region enlarged. Testes oval, symmetrical, behind ovary. Eggs very small. Adults parasitic in dermal cysts on abdominal surface of skin in birds.

What is probably the same species has also been found parasitic on sparrows at Boston, Mass. Its appearance is concurrent with periods of wet weather.

In 1902 MacCallum described an interesting parasite from the lungs and air passages of the river snapping turtle (*Chelydra serpentina*) found in Ontario, Canada. To this he gave the name of *Heronimus chelydrae*. In 1914 Barker and Parsons described a very similar form from the lungs of *Chrysemys marginata* taken in Minnesota, and also of various other turtles from Nebraska. This parasite they named *Aorchis extensus*. I have collected specimens from Illinois, Indiana, and Michigan of what is probably the same species. These two forms are so much alike that they may prove to be identical or at least to belong to the same genus, but they are in some respects very different from any other monostomes known, and I have established for them a new family with the following characters:

HERONIMIDAE Ward. Moderate sized monostomes with thick, elongate, soft body, slightly flattened, tapering toward both ends. Oral sucker weak, pharynx large, esophagus short or absent; ceca simple, narrow, extending to posterior tip but not united. Vitellaria compact tubular; uterus with four longitudinal regions; genital pore ventral to oral sucker, near anterior tip. Testis tubular, small, copulatory apparatus poorly developed. In lungs of turtles, northern North America.

Among the amphistomes Stunkard (1917) described a very peculiar form in which the oral sucker is subterminal and the acetabulum is divided by a transverse ridge into two pockets; this form which he named *Zygocotyle ceratosa* will not fit into any existing subfamily in the amphistome group, and for it must be made a new subfamily, the Zygocotylinae, which is characterized prominently by features just mentioned, and also by the lobed testis and the absence of a cirrus.

Among the distomes a number of changes seem necessary. The well-known parasite of native American herbivores, which was first named *Distoma magnum* by Bassi in 1875, has been included heretofore in the genus *Fasciola*, altho it has no distinct anterior cone, set off from the main part of the body, and the vitellaria are confined to the region ventral to the intestinal branches. The suggestion of Odhner that this form should be made a new genus seems thoroly justified by renewed study, and for it I propose the name *Fascioloides* with the type *Fascioloides magna* (Bassi 1875).

Among the Echinostomes a species from the loon (*Gavia imber*) and from Bonaparte's gull (*Larus philadelphia*) was described by Gilbert (1905) as *Echinostoma spinulosum* Rudolphi; it can not be that species. From Gilbert's description which is good tho not complete I regard it as a member of the genus *Stephanoprora* Odhner 1902 to which the name *Stephanoprora gilberti* is now given.

Much confusion has been introduced into the family of the Azygiidae by the formation of new genera for forms which are merely extreme types of the genus *Azygia*. This is a powerfully muscular distome and may be greatly distorted in the process of preservation. Specimens taken from a single host at the same time and preserved in the same way often present marked external differences in size and form. The genera *Megadistomum* of Leidy and Stafford, *Mimodistomum* of Leidy, and *Hassalius* of Goldberger are instances of such extreme specimens that really belong to the single genus *Azygia*. These genera must accordingly be suppressed. Altho many records were found of the occurrence in North America of the common European species *Azygia lucii* Müller (often wrongly called *A. tereticolle*), the study of a great number of supposed specimens from different localities and hosts has furnished no evidence of its presence here, and I regard the earlier records as erroneous and due to confusion with other native species.

Among those distomes of the Allocreadiidae which have a group of muscular papillae around the oral sucker, Stafford (1904) separated Lander's *Distoma petalosum* as a new genus *Acrodactyla* from *Crepidostomum* and *Bunodera*. The separation is justified, but the name proposed is preoccupied, and I have substituted *Acrolichanus* with the type species *A. petalosa* (Lander). Stafford states that this form which is common in the intestine of the Lake sturgeon (*Acipenser rubicundus*) in the Great Lakes and St. Lawrence river, is "on the authority of Looss the *D. auriculatum* Wedl of Linton." I am unable to accept this conclusion or the comment of Odhner that *A. petalosa* Lander is a synonym of *A. lintoni* Pratt. By the courtesy of C. H. Lander I have the original drawings of his form, as yet unpublished. A careful comparison of the details in the drawing with the evidence at hand on the other species noted is adequate to establish the distinctness of Lander's type. More data on this group will be published soon.

In 1910 H. L. Osborn established the genus *Cryptogonimus* to contain a species, *C. chyli*, he had found in the black bass and rock bass from Lake Chatauqua. The attempt to include this form in a systematic treatment of the distomes has necessitated forming a new subfamily for it, characterized as follows:

CRYPTOGONIMINAE Ward. Very small, spinous distomes, of uniform width, with bluntly rounded ends. Oral sucker relatively large and prominent. Ventral sucker double, minute, enclosed in pocket with genital pore between the two parts. Prepharynx, pharynx, and short esophagus present; crura extend to anterior margin of testes. Excretory vesicle Y-shaped, forking at oviduct, anterior branches reach to pharynx. Testes elongate, parallel, dorsal, in posterior third of body; seminal vesicle convoluted, prominent; no cirrus or sac. Ovary ventral, proximate to testes, slightly lobed. Vitellaria scanty, lateral, in central region of body. Uterus with descending ramus on right, slightly coiled, extending to posterior end, ascending ramus returning on left, crossing in front of ovary and passing on right to genital atrium. Eggs small, dark. In alimentary canal of fresh-water fish.

The location of this subfamily is uncertain, but wherever placed it is somewhat isolated. Odhner would include its type genus *Cryptogonimus* and also *Caecicola* in the *Acanthochasmidae*; in that event they both must be regarded as having lost the crown of spines characteristic of the family and are sufficiently distinct to justify the formation of separate subfamilies, at least for the genus *Cryptogonimus*.

Odhner (1910) has given a very careful analysis of a complex group of distomes which he names the *Lepodermatidae*. The family is, however, substantially equivalent to Lühe's *Plagiorchidae*, and while Odhner's emendations in the description should be accepted it seems wise to retain the earlier name. This very complex group is richly represented in the North American fauna and it is not unlikely that further study will show the need of splitting it up into two or more families. The precise structure of most North American representatives in the group is too poorly known to justify such a step at present. Among the genera included here are the frog lung flukes belonging to *Pneumonoeces*, recently worked over by Cort (1915). Looss has given such a thoro analysis of generic characters for these forms that one North American group must be separated as a new genus to which the name *Pneumobites* may be given; *Pn. longiplexus* (Stafford 1902) is the type of the genus. It is characterized by elongate lateral and nearly symmetrical testes, and lobed ovary in contrast with the round, median testes and entire ovary of *Pneumonoeces*. The forms are larger and thicker bodied than *Pneumonoeces* and the extra caecal longitudinal folds of the uterus are more pronounced, reaching nearly the length of the body. *Pneumobites breviplexus* belongs also in this new genus.

LARVAL STAGES OF TREMATODA

Some confusion has crept into the literature by virtue of inexact use of the terms employed to designate larval stages of trematodes. The cercaria is the youngest stage in the sexual generation and is produced in a redia or sporocyst; it has ordinarily a period of free existence and a caudal appendage used in locomotion. In exceptional cases the free stage is suppressed and the transfer is passive. The tail may

be lacking and to such forms the name cercariaeum has been given; this term is a convenient group designation to include all cercariae that are at birth tailless and does not properly embrace such as secondarily throw off the tail. The rejection of the tail regularly occurs when the larva encysts and at the same time there is discharged the secretion of the cystogenous glands which in many free cercariae are very conspicuous structures. These two changes mark the transition from the cercaria stage to the young or agamic distome, and after they have occurred the larva may no longer rightly be termed a cercaria. The use of the term encysted cercaria for this stage is confusing and should be discontinued; the collective name Agamodistomum was introduced by Stossich in 1892 for this condition. From this point the change into the adult distome is merely a process of growth in size and differentiation of the sex organs. The agamodistomum stage is often only transitory as when the cercaria introduced into the alimentary canal of a final host rejects the tail and enters at once on the growth period that yields the adult fluke; it may, however be encysted in the flesh of some secondary host in which relatively unchanged it awaits the transfer to a final host before the growth period sets in. There is, however, no morphological distinction between the larvae in these two instances and no call for separate designations. A review of the literature on North American trematodes shows many cases in which agamic distomes have been described as cercariae. The rectification of these errors will aid in the elucidation of the various life histories involved.

MORPHOLOGY OF NEMATODA

Among the Nematoda s. str. it has been the custom to recognize a number of groups of family rank, and no attempt has been made to ascertain the relations of these families to each other or to form of them higher groups. To be sure, some recent workers have exalted the families of earlier workers to superfamilies, and this change seems both advisable and calculated to aid in their more adequate interpretation, but they remain none the less isolated and unrelated subdivisions. I am of the opinion that more precise study of the morphology of these groups will furnish the basis for interpreting their relation to each other. In line with this I wish to call attention briefly to some results of morphological studies which I think serve to clear up the situation in part at least.

In describing nematodes, terms have been used loosely which should have a definite morphological significance, and the confused usage has served to conceal distinctions that exist clearly. One such case concerns the designation of the specialized region surrounding the mouth. All sorts of structures developed at this point are called lips, and various sorts of projections surrounding the buccal orifice are designated

a capsule. If one examines with care the oral armature one finds a number of distinct types of structure, each of which shows various modifications, but in most cases the fundamental type comes out clearly after study if not at first inspection. Three types which are easily distinguished I propose to call lips, jaws, and capsule, giving to each term a definiteness which accords with the condition in the old and well known examples of each and restricting each term to conditions which agree morphologically with the typical case.

True lips are best illustrated by *Ascaris* (Fig. 1). The anterior end of the body viewed *en face* shows three lobed projections which are varied in form and detail of structure in a manner characteristic of individual species, but which always hold the same relations to the planes of symmetry. One, the large lip, is dorsal, whereas two others, smaller, are ventral. That the large lip is to be interpreted as the fusion of two separate parts may be seen in the size, the two papillae it bears, and in other details of structure. This lip occupies the entire upper (dorsal) semicircle of the oral circumference and the line which separates it from the lower lips conforms to the lateral plane of symmetry. On the other hand the two inferior lips are clearly dextral and sinistral, being divided by a narrow slit that lies in the ventral half of the sagittal plane. These lips work as a three-parted organ, gripping rather weakly small objects that are drawn up between them. The orifice in this case is tripartite with the main axis lateral and the secondary axis extending ventral at right angles from the former.

True jaws are best illustrated by *Camallanus* (Fig. 3). The oral armature has only two distinct parts, and these are divided along the median line, the slit separating them being dorso-ventral and the parts symmetrical on the right and left. As seen in use this type of structure is distinctly a grasping organ; the parts move against each other with a powerful action and hold with vise-like grip. In the typical case, each part resembles in general appearance the shell of a *Pecten* and at the outer margin the two fit closely on each other. As the body of the nematode ordinarily lies on its side, such a structure may appear like a capsule, because the dorso-ventral slit is not apparent, but if the head is rotated carefully true jaws are easily distinguished from the true capsule — the type to be described next.

The oral capsule (Fig. 2) is spherical or cup-shaped, as seen best in the strongyles in which it presents great variety in individual details but a clear agreement thruout the group in fundamental features. At the anterior pole the sphere is cut off by a plane at right angles to the long axis of the worm so as to leave a circular orifice which is less frequently oval when the capsule is compressed laterally, but which is not a narrow dorso-ventral slit. The capsule is furthermore possessed of a considerable cavity which may be itself nearly spherical and which

in the higher or modified types carries on its inner wall cutting and piercing organs such as teeth, lancets, etc., as in the various hookworms. The oral margin of the capsule may be papillate, serrate, or ornamented by fine spines as in many sclerostomes.

Perhaps the most invariable and characteristic feature of the oral capsule proper is its rigidity; while the internal features impart to it a definite bilateral character, the external form is unspecialized or at most radial in type. Its function agrees fully with this. The capsule itself is immobile and works as a cupping or sucking organ; and the internal structures move and by piercing or tearing the tissue wall to which the cup is applied, release fluid materials or torn fragments of cellular character which are drawn down the esophagus to serve as nourishment.

Not all oral armatures described for nematodes can be reduced to these three types. In many cases the data are too general and inadequate to permit of any decision as to the fundamental plan of structure represented. More exact study of this region will result in demonstrating the morphological resemblance to the types described above of some mouth parts yet poorly known. Those forms in which the mouth parts are least differentiated are most difficult to interpret and classify. Perhaps it will be necessary to recognize an undifferentiated type with only a few papillae around the oral opening, and it may well be that further knowledge will justify the designation of still other types of oral armature. Meanwhile it is important in the interests of accuracy and clarity to keep at least these three or four types distinct and to examine as many nematodes as possible in order to determine how far they conform to the morphological plans described or depart from them. The exact application of this test in recent work (Ward and Magath, 1917) and in other cases yet unpublished has been of marked service in reaching conclusions as to the true relationships among the Nematoda.

Another morphological factor which deserves emphasis is the structure of the esophagus. The most common type is that seen in the ascarids. It is pronouncedly muscular in type, with the fibers transverse to the long axis of the organ and conspicuous on first examination as cross lines. This esophagus is tripartite in cross section and is a powerful pumping organ. (Fig. 11.)

A type of radically different character is the capillary esophagus long known and exploited as a diagnostic feature in the trichina and whipworm. It consists of a row of cells pierced thruout the entire length by a delicate tube of minute caliber. This tube has evidently no power of changing form or caliber in functioning and is a sucking organ fitted to the ingestion of fluid nourishment exclusively. The various nematodes which possess such a capillary esophagus I have

grouped together in a suborder, the Trichosyringata in contrast with those having a muscular esophagus which form the suborder, Myosyringata.

Among the Myosyringata one may observe some conspicuous modifications of the simple muscular esophagus just described. Possibly the most marked of these is the development of two specialized regions in the canal. The first is purely muscular and conforms to the simple muscular type except that it has no specialized region at the posterior end and is separated by the second region from the chyle stomach or intestine in which the process of digestion actually occurs. There is at most a line, partition, or constriction between the first or muscular region of the esophagus and the second. The latter is not uniform in appearance and may even be muscular in character like the first region. Usually, however, it is granular in appearance rather than striated and has more opaque walls. It terminates posteriorly in the valve or other special apparatus which marks the entrance into the intestine. Its function has not been clearly demonstrated, but it seems not to be a pumping organ.

In forms having a double esophagus various degrees of specialization may be noted. In the simplest case, *Haplonema* (Ward and Magath, 1917) one can see only a transverse partition (Fig. 12) dividing the esophagus into two regions which are both apparently muscular, but which differ in precise optical appearance so as to indicate functional differences between them. In other cases the distinction in histological character is more marked, but the separation between the two parts is not much more distinct. Finally, in *Camallanus* a deep constriction divides the anterior region very clearly from the posterior. In such cases the second part is easily overlooked and the description of the first region gives the worm an apparent likeness to the *Ascarid* type with a simple muscular esophagus. (Figs. 13, 14.)

One finds considerable range in the length, both absolute and relative, of the two regions of the esophagus. In the simplest case yet recognized (*Haplonema*) the total of both regions is not more than the simple muscular esophagus, but in the forms like *Camallanus* each region is so long that together both constitute a conspicuous part of the total length of the worm.

The double esophagus is one of the most characteristic features in the structure of nematodes included in the Spiruroidea and its occurrence may be confined to that group exclusively.

Some confusion also exists in description of the structure of the specialized caudal end in the male because of indefiniteness in the use of terms. In many males one finds lateral cuticular expansions about the caudal end which are utilized as grasping organs in copulation. The term bursa has been often used for all these organs; one may,

however, early recognize at least two types that are morphologically distinct, and that may well receive different names. In one type the organ consists of semicircular expansions that include the extreme posterior tip of the body, joining behind it and indicating the median line by a deep notch or furrow where the two folds come together. The organ shows a series of lines or bands that radiate like the sticks of a fan from a basal point on each side, diverging as they approach the periphery of the fold. This organ which I propose to call the bursa is shaped like a shallow cup or saucer and forms a conspicuous sucker-like termination for the caudal end of the male in the true Strongyloidea. (Figs. 8-10.)

The second type often resembles the first in a superficial way, but on more particular examination shows clear differences. The cuticular expansions are narrow linear folds; they extend along the sides of the body for some distance anterior to the caudal tip, but do not reach posteriad beyond the tip. The outer margin of the fold is nearly parallel to the body, but approaches it slowly, since the fold is broadest near its anterior end and tapers to zero between the anus and the posterior tip of the body. These folds possess bands that are generally speaking perpendicular to the long axis of the body, being parallel to each other and not radiating from a common center. These folds are not often broader than the body and may be so very narrow that they are easily overlooked. They may be called alae or wings, and constitute a simpler or less highly specialized type of clasping apparatus than the circular bursate type. The alate type is common and very likely characteristic among the Spiruroidea. (Figs. 6, 7.)

There certainly are other types of cuticular grasping organs among nematodes such as those of the trichina and the funnel-shaped organs of Eustrongylides and Hystriechus. But I have not yet had opportunity to study these personally, and do not desire to do more than mention them here.

Among the Acanthocephala the simple forms which have in the hypoderm and lemnisci only a few giant nuclei constitute a group sufficiently distinct from other types to be ranked as a family which I propose to call the Neoechinorhynchidae with Neoechinorhynchus Stiles and Hassall 1905 as the type genus. The family may be characterized as follows:

NEOECHINORHYNCHIDAE Ward. Acanthocephala with hypoderm consisting of a syncytium in which are six giant nuclei, ordinarily arranged so that five lie in the mid-dorsal line and one in the mid-ventral. One lemniscus contains two giant nuclei and the other only one. These nuclei are usually conspicuous on external examination. The proboscis sheath contains only a single layer of muscles. The cement gland is a compact mass. A neck is lacking. The muscles are weakly developed. The lacunar system is supplied only with simple circular connections.

The type genus of this family has been very fully and accurately described by Van Cleave (1913). He ranked in this genus one species which differs from all others in it in having an elongate proboscis with numerous irregular circles of hooks in the place of the globose proboscis with only three circles of hooks. For this aberrant form I have established the new genus *Tanaorhamphus* with *T. longirostris* (Van Cleave 1913) as type. The extreme length of the proboscis and the large number of hooks distinguish these forms at sight from those of the genus *Neoechinorhynchus*. Of other points of difference in structure perhaps the most striking is the constant presence of 16 nuclei in the cement gland of *Tanaorhamphus* where *Neoechinorhynchus* has only 8. One notes also that in *Tanaorhamphus* the hooks of the anterior row are not conspicuously larger than those following, but in *Neoechinorhynchus* the difference in size is real in all and very marked in most species.

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EXPLANATION OF PLATE

Figs. 1 to 4.—Apical views of anterior end to illustrate different morphological types among Nematode heads.

Fig. 1.—True lips; tripartite arrangement, in which, however, the dorsal lip has two papillae. *Ascaris lumbricoides*. (After Leuckart.)

Fig. 2.—Oral capsule; orifice an undivided circle surrounded by six equidistant papillae and showing tripartite esophagus inside and below level of capsule. *Cylichnostomum coronatum*. (After Looss.)

Fig. 3.—True jaws; bilateral type with mouth a dorsi-ventral slit. *Camallanus ancyloides*. (Original.)

Fig. 4.—Radial arrangement; six small protuberances of irregular form, often called lips and jaws but evidently not equivalent morphologically to structures shown in Figures 1 and 3. Note however, the four papillae. *Protospirura muris*. (After Schneider.)

Fig. 5.—True capsule but bent 60° dorsad. Longisection of anterior end to show dorsal (secondary) position of mouth in *Ancylostoma duodenale*. (From Neumann and Mayer after Brumpt.)

Figs. 6, 7.—Posterior end of male showing true alae in ventral aspect. Papillae or ribs shown in outline only.

Fig. 6.—Alae joined at anterior limit. *Physaloptera muris-braziliensis*. (From Hall after von Drasche.)

Fig. 7.—Alae narrow and not joined. *Spiroptera penihamata*. (After von Drasche.)

Figs. 8-10.—Posterior end of male showing true bursa. Rays of bursa shown in outline.

Fig. 8.—Bursa with distinct, well separated lobes, from dorsal aspect. *Haemonchus contortus*. (After Ransom.)

Fig. 9.—Bursa with slight median notch between lobes. *Heligmosomum minutum*. (From Hall after von Linstow.)

Fig. 10.—Lobes completely fused along median line to form a single organ. *Oesophagostomum columbianum*. (After Ransom.)

Figs. 11 and 14.—Dorsal views of anterior end to illustrate various types of esophagus among nematodes.

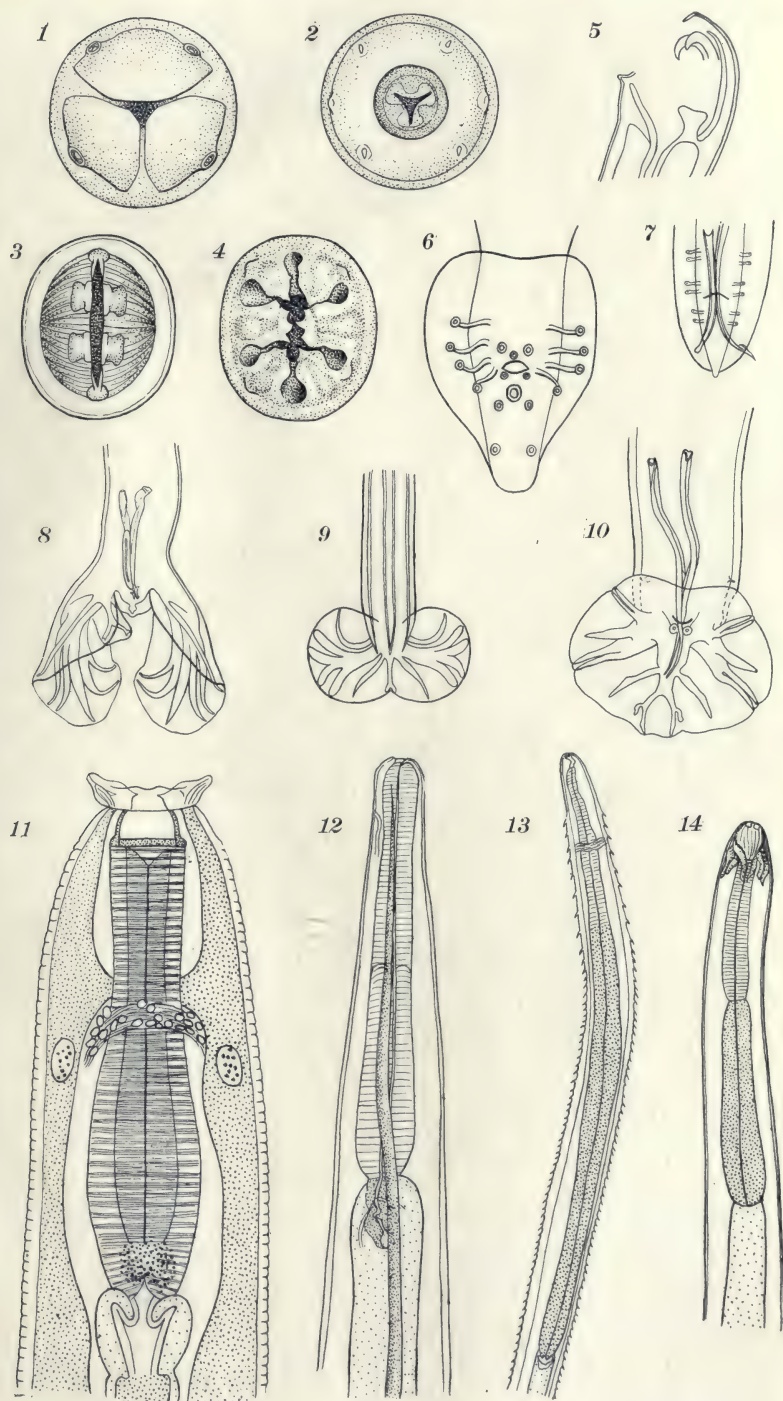
Fig. 11.—Muscular esophagus with single region only. Note, however, granular (glandular?) mass close to posterior end. *Cylichnostomum auriculatum*. (After Looss.)

Fig. 12.—Muscular esophagus divided by distinct transverse partition near center of length. *Haplonema immutatum*. (After Ward and Magath.)

Fig. 13.—Esophagus with anterior muscular and posterior granular regions clearly distinguishable but not separated by partition from each other. *Spinitectus gracilis*. (Original.)

Fig. 14.—Esophagus with two regions, viz. anterior muscular and posterior granular, sharply separated by constriction and transverse partition. *Camallanus oxycephalus*. (Original.)

WARD—NORTH AMERICAN PARASITIC WORMS





ON THE SPOROZOON PARASITES OF THE FISHES OF WOODS HOLE AND VICINITY

III. ON THE CHLOROMYXUM CLUPEIDAE OF CLUPEA HARENGUS (YOUNG), POMOLOBUS PSEUDOHARENGUS (YOUNG), AND P. AESTIVALIS (YOUNG)

C. W. HAHN

The attention of the writer was drawn to some very common white pseudocysts in the body muscle of the young herring by Dr. Edwin Linton in July, 1910. He had previously (1891) identified the contents of these cysts as myxospores of a myxosporidian. An effort was made at once to trace the life-history of this organism, also to learn its method of infection, the pathological condition induced, and the effect upon the vitality of the host. Several hundred herring, ranging in size from $1\frac{1}{2}$ to 5 or 6 inches have been examined. About 54 per cent of them had buried in the body muscle clusters of myxospores (pseudocysts) large enough to be visible to the unaided eye. These spores are brought to light by cutting the flesh lengthwise of the body on each side of the backbone. As will be evident after reading the following pages, it is certain that a microscopic examination of fish in which no pseudocysts can be seen by ocular examination, would greatly raise the proportion of fish which harbor such parasites. Only small fish under 4 or 5 inches are known to be infected.

The pseudocysts are sometimes as large as a grain of wheat. They are usually white or cream colored, soft or creamy in structure, and spindle shaped, especially when small. Small pseudocysts cannot be distinguished at first sight from worm cysts, but the latter, when pressed with the tip of a scalpel, resist and regain their shape when the pressure is withdrawn. The cysts mash up just like a bit of soft cheese. Usually the pseudocysts lie between the bundles of fibers. Large masses occur in pockets just beneath the integument, which is slightly mounded over them. A pin-prick brings forth a pus-like fluid. The large cysts appear to make their way from deep-seated positions to the surface. A small hole then forms in the integument, through which the mass escapes. No case of the complete discharge of myxospores from such a pore has yet been observed. The process has been observed in its initial stages, and many cases have been observed of worm cysts which were just escaping or had just left pores identical with those just described.

The pseudocysts of this *Chloromyxum* occur throughout the body musculature. It is very common to find several just at the base of the caudal fin rays. They are also frequent just posterior to the skull and branchial cavity. It is remarkable that a fish can retain life with its flesh so burdened with the cysts, in some cases so abundant that it is impossible to count them. The large ones coalesce and form huge cavities filled with a pus-like fluid. A fish an inch and a half long may contain several hundred pseudocysts and continue for a time to hold its place in a school of several hundred fish against the incessant attack of numerous enemies.

The pseudocysts are composed almost entirely of mature myxospores. When muscle tissue adjacent to the cysts is examined under the microscope, it is found to contain myxospores in masses of all sizes and numerous isolated myxospores or chains of spores between the fibrillae. These aggregations of myxospores vary in size from one spore to two or three times the size of a grain of wheat. The shape is determined by physical conditions. In small cysts it results in long or fat oat-shaped structures.

It is not possible by direct observation to attribute any evil effects upon the host to the presence of these numerous passive pseudocysts. The fish give no visible evidence of inconvenience. But when one takes into consideration the ravages of the trophic stages which must have preceded the harmless myxospores and the toxic substances secreted during the process of sporogenesis, it is very probable that some considerable injury has already been inflicted upon the host before the myxospores develop. My statistical studies prove conclusively that the pseudocysts are in reality more or less injurious.

The trophic stages of the gall-inhabiting species of this genus are known through the researches of Erdman (1910), Auerbach, and Léger (1906). No reference to the multiplicative stages of flesh-inhabiting species has yet been found. The occurrence of myxoplasms in both inter- and intra-cellular positions in muscle tissue has been described by Thélohan (1891, 1893) for *Glugea destruens* Thél. in *Callionymus lyra* and by Gurley (1893) for *Pleistophora typicalis* Gurley in *Cottus scorpius*, and by both Pfeiffer (1891) and Keysseltz (1908) for *M. pfeifferi* in the barbel. The pathological conditions in most of these cases are practically identical to those which I have found in *Funulus*. As for details regarding the trophic stages of the parasites themselves, they are scanty and cannot be satisfactorily correlated with life histories as they are now known.

The origin of the multiplicative trophoplasts is still somewhat obscure. It is probable that large myxoplasts like that shown in Figure 15 undergo schizogony and set free the small trophoplasts which

occur so abundantly in newly infected tissues. The smallest of these is about 2μ in diameter. They are very closely scattered in patches throughout the myoplasm of infected muscle fibers, sometimes so close together as to be almost in contact. The fact that the size is not uniform would lead to the conclusion that they multiply by fission, since it is the habit of most myxosporidia to advance from stage to stage simultaneously. But one never meets with couplets in the same cavity in the myoplasm, as would be the case if fission were common.

The distribution of the small and large trophoplasts is very irregular, there being frequent isolated individuals. The muscles of the head region, especially those around the branchial arches and the eye and jaw muscles, are frequently riddled with these parasites. They also occur abundantly in the striated muscle of the digestive tract (the herring has ribbon-shaped striated fibers in the wall of the intestines) and less frequently in the body muscle near the backbone. They are both inter- and intra-cellular (Fig. 8).

When fresh muscle is examined, the trophoplasts appear as almost invisible homogeneous droplets. Stains have no greater tendency to take hold of them than they do the trophoplasts of *M. musculi*. Anilin stains reveal them as white spaces in the myoplasm. Hematein gives to them a homogeneous clouded appearance as shown in Figure 13. The shape in the very small individuals is rounded or ovate. Larger ones are irregular in shape, but in fixed preparations have always an entire contour. Rarely a medium-sized trophoplast has the nucleus faintly stained (Fig. 13).

There is the same evidence that these bodies are not artifacts that has been given for the trophoplasts of *M. musculi*. The appearance of the older stages of the multiplicative trophoplasts is almost identical with that of the young. But the size, shape, and distribution gradually change. Some are evidently motile, judging from their shape (Fig. 13). A few individuals of this type occur in rather isolated positions. The muscle is taken from the body near the backbone. Adjacent tissues are liberally sprinkled with smaller trophoplasts. Older stages than that in Figure 13 are usually still more isolated from the more gregarious young stages. It is probable that the schizogony sets free innumerable multiplicative spores which throughout their growth migrate from atrophied toward normal tissue and become quiescent in the mature schizont (Fig. 15). The latter are very large (sometimes 40 to 50 by 50 to 60μ), and almost spherical in form. They are usually in tissues which are comparatively free from trophoplasts of small size, and also free from evidences of infection. Rarely one can discern a faintly stained material within which is probably the nucleus. Another large body which is almost identical in appearance, but which is not asso-

ciated with the smaller trophoplasts, nor connected with them by any link as yet discovered, is of much greater size. It reaches 890μ in length by 30μ in width. The outline is sharp and the slightly opaque cytoplasm reveals no trace of the internal structure. These bodies lie between the muscle fibers, sometimes in rows. Around and between them is a granular deposit which runs into the masses forming faint partitions. It gives the appearance of a large number of schizonts which have more or less fused. Separate large myxoplasts do occur in exactly similar positions. This form and position in the muscle fibers is also reproduced in a striking way by masses of sporoblasts and myxospores in tissues which lack all of these earlier stages. It is therefore apparent that the large elongated myxoplasts represent aggregations of some kind of migratory trophoplasts. That they do not grow in situ is shown by the uninjured condition of the tissues. They are too large to represent the adult of any single one of the largest myxoplasts without a vigorous consumption of host tissue of which there is negative evidence. The sporoblasts and myxospores occur in chains and smaller groups such as to indicate that there are numerous small clusters of sporoblasts which are not gathered together as in the cases above cited. It is altogether probable that either mechanically or through their own activity, the propagative myxoplasts, having migrated deeper into the tissues of the host, become assembled into larger or smaller groups. In this condition the sporoblast cells are formed and sporulation takes place.

In these two kinds of presporulating cells one evidently has the multiplicative and propagative schizonts. The former represented in Figure 15, is always found in tissues adjacent to the young multiplicative trophoplasts. The latter is never to be found in tissues which contain multiplicative stages, but always in the very presence of sporoblasts and myxospores. The multiplicative stages and schizonts alone are encountered in the muscle of the digestive tract and its vicinity. The propagative schizonts are always in the tissues of the body muscle. These facts prove (1) that the trophoplasts migrate from centers of infection to parts free from previous attack; (2) that the general trend of the migration is from the digestive tract into the body muscle. (3) that the initial infection takes place through the digestive tract. It is probable that this occurs throughout the entire length of the digestive tube, because there is no very marked superiority in the number of myxospore cysts of the anterior body muscle over the posterior region. However, this equality of distribution may be due to transit through the blood vessels.

In the large schizont cysts described above one can occasionally find the contents divided into hundreds of irregular-shaped cells whose

cytoplasm is so clear and structureless that the cell boundaries are almost invisible. They contain conspicuous masses of varying size and shape and intensity of stain. The two upper cells of Figure 1 are almost identical with the above, but were drawn from a group that had been set free from the cyst. These are sporocysts. The deeply stained portion represents the developing myxospores and is not a nucleus as one might suppose.

From the above and further details of sporogenesis which follow, it will be seen that sporoblasts may not necessarily occur free, the large presporulating masses being composed of many assembled myxoplasts of comparatively large size. Within the schizont, there are no doubt many stages of sporogenesis as yet concealed because of inability to stain them. While it is known that the sporocysts arise from some sort of sporoblast or gametoblast cells, the method of origin of said cells is absolutely unknown, whether it be by a continuous process of internal budding or a simultaneous schizogony.

The earliest condition of the spore which I am able to identify with any degree of certainty is shown in Figure 16. It is a sporocyst composed of cytoplasm that is identical in properties to that of the multiplicative trophoplasts. The nuclei do not stain. What appears to be a large nucleus at the center is really the early condition of a myxospore. It is irregular in shape and at first discloses no nuclei. Sometimes these spore fundamentals are encountered free from the sporocystplasm as shown above in Figure 16. The rectangular form of the myxospore is assumed later (Fig. 1).

In the homogeneous stainable portion of the sporoblast which later becomes the myxospore, there at first appears a large, more densely staining portion, which, by its behavior, proves to be the nucleus (Fig. 1, upper sporoblast). The nucleus becomes more concentrated (the two lower left-hand cases), and by some method of fission not yet clear, it is divided into as many as nine fragments (Fig. 11). In some cases the sporoblasts contain all of the nine nuclei before there is any evidence of polar capsules. In others the polar capsules appear in the presence of only four or five nuclei (Fig. 1, the right-hand sporoblast). Myxospores with one nucleus opposite the large end of each polar capsule are very common. The others may occupy almost any position in the free periphery of the sporoplasm. In Figure 11 there are two nuclei opposite each polar capsule. Five of these are the generative and wall nuclei which have not yet left their central position. With some stains the polar capsules are conspicuous and the nuclei almost invisible (Fig. 10).

The mature myxospore (Fig. 10) is more or less square with bulging sides. The polar capsules are pointed at the inner end and have a

very short, tapered neck. The myxospores of *C. clupeiidae* measure on an average 7μ across from one side to the other. The polar capsules are a trifle over 1μ in diameter and about 2μ in length. I have examined many hundreds of these myxospores and have never discovered any indication of valves in the spore wall.

When compared with the *C. funduli* (Hahn, 1913: 205) it is readily distinguished. The latter is circular when viewed from the polar end and tapers with an incurved outline from the antipolar region to the polar end. The polar capsules are therefore drawn out and curved to correspond to the exterior. In *C. clupeiidae* the profile from the polar end is square with rounded corners. It is not drawn out at all on the polar end, but is shaped like a very low conical pyramid with a round base. The profile is, therefore, that of a hemisphere on the polar side and of an oblate spheroid on the antipolar side.

As far as I have been able to discover, there is no *Chloromyxum* answering to the above description which has previously been described, unless it is *C. quadratum* from the muscles of *Callionymus lyra* (Thélohan, 1895). This is a very similar parasite, though by no means identical. It forms small pseudocysts and masses of myxospores in the muscles. The myxospores occur in small groups or bundles of ten to twelve which are massed together into secondary groups of three to thirty or more. The primary groups of myxospores are probably derived from a single propagative myxoplast. The propagative myxoplasts, having been gathered into masses are thus responsible for the primary and secondary groups above mentioned. No such limitation of myxospore groups has been observed in *C. clupeiidae*. Otherwise the conditions of spore formation are apparently the same.

The myxospores of *C. quadratum* are much longer than those of *C. clupeiidae*, being 7μ in length along the polar axis and 5μ in diameter, while the myxospore of *C. clupeiidae* is 7μ in diameter and not over 5μ in length. *C. quadratum* is deeply incurved on the sides and has a long polar apex with very small polar capsules.

The myxospore of *C. mucronatum* (Gurley, 1893) differs in shape from *C. clupeiidae* in a very distinctive way. The profile, as seen from the polar end, is similar to that of the latter, but is circular in outline. The profile from the view at right angles to the polar axis is relatively shorter in *C. clupeiidae* than in *C. mucronatum*, otherwise they are very similar. The polar capsules of the latter are relatively a little smaller and shorter. The difference between published figures of the two species may be due to a difference in relative maturity, but *C. mucronatum* is a free-living form from the gall and is polysporous.

The most obvious pathological change which is induced by the *C. clupeiidae* is the degeneration of the muscle fibers. As in the inva-

sion of fundulus muscle by *M. muscoli*, the early trophic stages cause the myoplasm to hypertrophy. But I have never encountered tissues in the herring that had suffered in a way comparable to those of Fundulus. Parts of the musculature and connective tissue of the intestines of the former are completely disintegrated, while the parasites occur in herring flesh by hundreds of thousands; no gross hypertrophy is ever to be observed. In the muscle of the head the fibers are sometimes riddled with holes containing the parasites. Atrophied muscle fibers are also to be encountered in the body muscle. As such fibers occur in tissues having only multiplicative stages, it is quite certain that the greatest injuries to the body muscle are not caused by the propagative stages. This conclusion is confirmed by the location and habits of the propagative stages themselves.

Because of the pathological condition one finds in the muscle fibers of infected herring, one would expect this disease to be very destructive to the fish. But when caught the fish are in apparent good health. The enormous masses of pseudocysts in the flesh do not inconvenience the locomotion of the herring so far as one can observe by watching schools of young herring as they dart about escaping from their enemies above and below. However, weak and unfit fish would undoubtedly be overtaken by such swift enemies as squid, mackerel, bonito, etc., with which they are constantly beset in the open sea. Those which were severely injured by the multiplicative stages have no doubt already been eliminated from the schools one observes in open water, the survivors having myxospores only. One can only speculate upon the possible mortality of a disease which, having passed through its most virulent stage, leaves considerably over 50 per cent of the survivors infected. Additional observations along this line will appear in a later paper.

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ENDAMOEBA BUCCALIS

II. ITS REACTIONS AND FOOD-TAKING

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The material for the present paper was collected from the same source as that of the previous study — a single host and a single point of infection, an upper premolar tooth. For details of preparation of material and method of study see Nowlin (1917).

MORPHOLOGY AND BEHAVIOR

The size of the living trophozoite found in smears varies from about 12 to 40 μ when in a spherical condition, and the latter may elongate to 80 μ . The amoebae most frequently encountered are from 25 to 35 μ , constantly forming pseudopodia if it be no more than shoving out a slight rim on one side and withdrawing it. A specimen just out of the mouth is usually active unless it has been chilled or is forming a cyst. It is not necessarily progressing, but can be seen to change shape rapidly by sending out blisters of ectosarc one-third its entire size. One of these rapidly melts into another and will continue to do so unabated for an hour or more if conditions of warmth and moisture are favorable. A group of twenty amoebae massed into a clump were observed vigorously crawling over each other for more than an hour. Food material was the suspected stimulus for such motion, but when they finally separated from a sudden cooling of the slide, no food was visible. They might have exhausted any supply, but more probably they hung together thru positive thigmotaxis.

During progressive movement the endamoeba is an interesting example of the highest development of pseudopodial motion. The animal is elongated to about the proportions of a thumb, and clearly differentiated as to ends. Forward is a clear protrusion of ectoplasm nearly half the length of the body, and rounded out like a bag. At the posterior end is a knob (Fig. 1a), which, if torn from an attachment will have little papillae or beads on the periphery (Fig. 1c). These threads of attachment enable an amoeba to carry about with it great masses of leukocytes and debris. It exhibits in this most remarkable strength for a one-celled organism. One amoeba can break up a large solid-looking mass of leukocytes by crowding into them. It can load up with a great cargo of cells and tartar which it carries over the slide as long as one has the patience to watch; nor does the load seem to impede its progress.

The very active movements of *Endamoeba buccalis* in smear preparations suggest great possibilities of damage in the gum by purely mechanical processes, tho this is by no means their full capacity for mischief, as will be seen under methods of food-getting.

Reactions to Light and Heat.—*Endamoeba buccalis* exhibits a much more marked positive thigmotaxis than do free living amoebae. To leave deliberately a clump of leukocytes to which it has been attached and venture into the open requires several attempts. In one case under observation there was a gap a little greater than the width of the endamoeba's body to be crossed before more leukocytes could be reached. The animal extended itself probably twenty times toward the mass, before it relinquished hold, and even then only after it had succeeded by an extraordinary stretch in touching the other side. These parasites have the capacity of stringing a pseudopod to a length of five or six times their body diameter. Attached thus an animal may

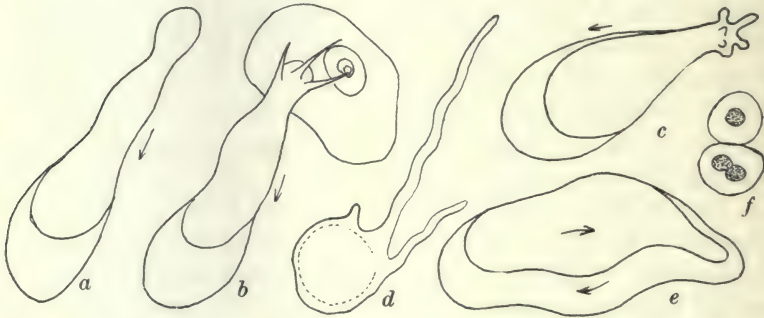


Fig. 1.—*Endamoeba buccalis* in movement. For details see text.

wander into a clear field, breaking the thread only when it comes in contact with a solid, or settles down for attachment to the slide. Many amoebae appearing free are found to be thus attached if the light is properly adjusted. Again the pseudopodia may assume a perfect corkscrew appearance, attached to the spherical body of the parasite and moving in various directions like waving tentacles (Fig. 1d).

The few experiments performed to test their sensitiveness to light suggest that they have lost this quality. No difference in behavior could be detected in very bright light and in reduced rays. They are very sensitive to heat, however, and where that is combined with light, as is often the case, there is marked reaction, as shown by increase of activity so long as that temperature is kept around body warmth.

No contractile vacuole is present, and only twice in the living specimens have anything like nuclei been observed. One morning every living amoeba in the smears, and many were made, showed a single red spot about the size of the nucleus of stained specimens. The host

had gargled with glycothymoline and that was suspected as the staining agent, but it could never be demonstrated again either by direct application or by gargling. Under one other condition a spot resembling the nucleus showed up uniformly in many living forms which had been kept sealed for six hours under a cover-slip. These endamoebæ assumed an encysted form without completing it with the outer wall, lost all their vacuoles, and showed a single dense spot slightly to one side of the center. This may have been a nucleus.

Food and Food Habits.—The adult or mature trophozoite form has from one to twenty food vacuoles. The usual appearance is three or four of these, each about one-fourth the diameter of the amoeba, and many smaller ones. In life, the contents of these large vacuoles are homogeneous spherical masses, having a greenish gray color, and showing only a single mass to a vacuole (Figs. 2*a* and *b*). In stained specimens also the food vacuole contents are solid masses, varying

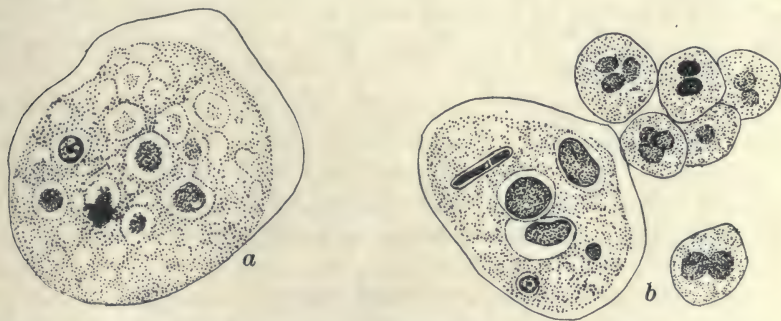


Fig. 2.—*a*, *Endamoeba buccalis* stained with Dobell to show nucleus. *b*, Stained as above to show food vacuoles and attached leucocytes.

only in intensity of coloring, and showing never any resemblance to leukocytes. They appear more as globules of fluid. No endamoebæ under observation during this study, though they have been watched carefully for this, have been seen to ingest leukocytes, red blood cells, as described by other writers, notably Smith and Barrett (1915), or anything solid except bacteria, and these in small numbers. That they never do this, I cannot say definitely, but I believe the following behavior may have been interpreted as ingestion.

The endamoebæ frequently attach themselves to leukocytes (Fig. 2*b*). I have seen them creep over the leukocyte and practically surround it until it looked quite like ingestion. A leukocyte will even become sufficiently incorporated to be taken away with the endamoebæ for a short time, but if watched sufficiently long the leukocyte is invariably left on the slide, apparently unharmed. I have seen other clear refractive bodies taken in like this and soon discharged, never becoming real food vacuoles.

SUMMARY

I conclude from my observations that *Endamoeba buccalis* absorbs its food mainly, taking in by osmosis the fluids of leukocytes or other media on which it rests, stores these colloidal substances in vacuoles, and by secretion of its own enzymes assimilates these as needed.

The reasons against believing that large food vacuoles are ingested leukocytes may be summarized thus:

(1) There is never but one body to a vacuole, while most leukocytes have one to three nuclei.

(2) There is never any granular area around the vacuolar inclusions, as would be the case if the cytoplasm of a leukocyte were ingested.

(3) Leukocytes have been surrounded by amoebae, but never ingested, according to my observations.

(4) The whole system of vacuoles can vanish from an *Endamoeba buccalis* exposed to unfavorable conditions, sooner than would be possible if these were solid inclusions; moreover, the leukocytes outside the endamoeba are left intact.

This method of food-getting by absorption would explain the shrinkage of gums where *Endamoeba buccalis* is present. I have seen no evidence of their penetrating epithelial cells, but there is abundant evidence that they draw supplies by applying themselves to the surface of tissues and by crowding between them (Fig. 2).

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THE CENTRAL NERVOUS SYSTEM OF THE PARASITIC ISOPOD, GRAPSICEPHON

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Some specimens of the genus *Grapsicephon* of the Bopyridae were obtained from the gill chambers of the common shore crab of Laguna Beach, *Pachygrapsus crassipes* Rand. One of these was sent to the United States National Museum and there determined to be of the genus here given.

Two specimens were sectioned and mounted in series; one was stained in carmine and one in hematoxylin. Only in the latter specimen was the poorly developed nervous system distinguished easily from the surrounding tissues. No supraesophageal ganglion was found and the ventral chain of ganglia was imperfectly developed. The whole central nervous system does not exceed one millimeter in length, or a little less than one twelfth the length of the animal. A wax reconstruction was made of the central nervous system showing the locations of the cellular areas.

There are at least four ganglia represented in the nervous system, but these are very imperfect and irregular ganglia. Beginning at the cephalic end the ganglion is quite well fused and occupies one third the whole length with no branches for some distance; then there are large irregular branches extending laterad. Next there is a division into something like connectives and other branches extending laterad, although these do not show well in the model, because they seem fused with the other parts. Near the caudal end of the ganglionic mass there are other divisions into connectives and near these, short branches. Altogether, there are six very irregular pairs of lateral branches which could be followed only for a short distance from the central nervous system, and four branches which arise from the caudal end.

The distribution of cells is on the whole much like that of other arthropods. Most of the cells are ventral in position, but irregular masses are seen at places on the dorsal side. The cells in many cases seem but poorly developed; the nuclei in some cases are like those of nerve cells, but most of them appear like poorly preserved material, although the general preservation of all parts of the specimen except this was very good.

In conclusion, it might be said that the animal has a degenerated central nervous system with indications of at least four ventral fused

ganglia. Branches are not perfectly formed and cannot be traced very far. Although there were a few striated muscle fibers in the animals, the movements of the living forms were very slight. If there is a dorsal ganglion it is so poorly differentiated as to be indistinguishable from the other tissues of the animal.

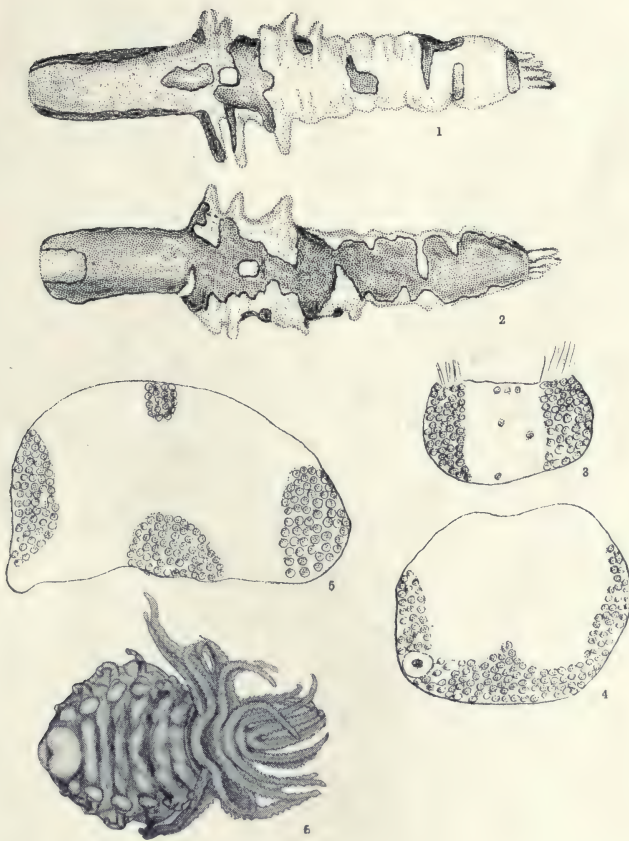
EXPLANATION OF PLATE

Fig. 1.—Drawing of a model of the nervous system of *Grapsicephon*, from the dorsal side, showing the cell areas in the more deeply shaded portions. The cephalic end is at the left. $\times 80$.

Fig. 2.—Drawing of a model of the nervous system of *Grapsicephon*, from the ventral side, showing cell areas by more deeply shaded regions. The cephalic end is at the left.

Figs. 3, 4, and 5.—Sections through various levels of *Grapsicephon*, central nervous system. The dorsal side is uppermost. $\times 300$.

Fig. 6.—Surface view of the whole body of *Grapsicephon*. Drawing by Harry Staples. $\times 3$.





INVESTIGATIONS OF THE VALUE OF NITROBENZOL AS A PARASITICIDE WITH NOTES ON ITS USE IN COLLECTING EXTERNAL PARASITES

WALLACE L. CHANDLER

Incident to the recent appearance of an article (Moore, 1916) recommending as a regular procedure the fumigation of animals with *nitrobenzol* for the control of their external parasites, a great deal of interest was aroused in the apparent possibilities offered by the use of this drug in various phases of parasitological work. A number of experiments, both field and laboratory, involving its use were initiated by the Department of Entomology of Cornell University.

At the outset it appeared to those more familiar with the chemical nature and toxic properties of nitrobenzol, that it would be highly desirable to do some definite experimental work to determine the physiological action of this drug on animals when administered by vapor inhalation. There are on record in the Index Medicus (see nitrobenzol, nitrobenzene, oil of mirbane, etc.) a great number of reports of fatal cases of nitrobenzol poisoning in man resulting either from the ingestion of the liquid or absorption through the skin. A few instances of poisoning by inhaling the vapor are also recorded. There have also been done a few careful experiments upon laboratory animals (Filehne, 1878) in which the drug was administered in the liquid form by introducing it into the stomach through a tube and by intravenous injections; and in these experiments symptoms of nitrobenzol poisoning with fatal terminations are described after the administration of even small doses. So far as can be determined, however, no satisfactory experiments are recorded in which the drug was administered by vapor inhalation.

In view of this situation, Dr. M. Dresbach and the writer undertook investigations to determine the action of nitrobenzol upon various animals when such animals were exposed to the vapor of this drug at various temperatures and for various periods of time. The experiments have now been in progress for more than a year and are being conducted at the physiological laboratory of the Cornell University Medical College. The facilities and resources of this laboratory have been placed freely at our disposal. We have also received material assistance and valuable advice from members of the staffs of the

departments of entomology, sanitary chemistry, physical chemistry and histology.

An attempt was made to simulate as far as possible the conditions recommended for the "fumigation of animals to destroy their external parasites." However, the necessity of reducing to a minimum all possible interfering factors, such as impurities in the nitrobenzol, excess of carbonic acid, excess of moisture, and variations in temperature was early recognized. Practically pure nitrobenzol was obtained by redistilling the commercial liquid until a product was obtained which proved experimentally to have a boiling point of 210.9° C. and a freezing point of 5.7° C., the boiling point and freezing point, respectively, of nitrobenzol (Landolt and Börnstein, 1912). Provisions were made in the construction of the apparatus for controlling the other possible interfering factors mentioned.

A detailed description of all the apparatus used will appear in a more technical paper dealing with this subject. In brief, the principal parts of the apparatus were as follows: A paraffin-lined metal tank of 40 cubic feet capacity, so constructed that it could be hermetically sealed with paraffin* and provided with glass windows in top and side through which observations of the temperature of the tank and the animal's condition could be made. In the center of the tank was firmly suspended a wire cage which served to protect the following: A triangular piece of linen, one end of which dipped into a container of nitrobenzol; a thermometer, and a small fan which was kept revolving at a rate sufficient to insure a homogeneous mixture of the air and other gases within the tank. A removable false bottom of wire netting served to keep the animal from contact with its excretions.

The procedure in any single experiment was as follows: The tank was freed from moisture, sufficiently for all practical purposes, by allowing plates of concentrated sulphuric acid to stand in it for a day or so, the tank being sealed. The sulphuric acid was then removed, nitrobenzol introduced into the container, the tank sealed again, the fan started, and a constant temperature maintained. After sufficient time had elapsed to insure complete saturation with nitrobenzol (this was shown experimentally to require several hours), the animal was quickly introduced, the tank sealed, and air saturated with nitrobenzol passed into the tank at a rate sufficient to insure aeration. An outlet was provided so that the air in the tank remained at atmospheric pressure. Observations were recorded as a rule every fifteen minutes.

The results of these experiments, obtained from observations made on a large number of dogs, cats, rabbits, rats, mice, guinea-pigs,

* Paraffin was found to be the best substance for this purpose since it is neither attacked by nor permeable to nitrobenzol.

chickens, pigeons, and frogs, are being compiled for publication in a more extensive paper; but it may not be out of place here to state that in all of the cases observed nitrobenzol has a serious poisonous action when administered by vapor inhalation. The intensity of its action, the type of symptoms produced, and the time elapsing from the moment of fumigation to the first onset of the symptoms vary greatly with different species of animals and even with animals of the same species. The dominant symptoms in all animals seem to be profuse salivation (in dogs, cats, etc., vomiting), diarrhoea, loss of coordination of the voluntary muscles, particularly those of the extremities, and a general loss of muscular tonus. In dogs, guinea-pigs, and poultry muscular tremors were observed. There were also generally present long periods of clonic convulsions ending in tonic convulsions involving the whole body and followed by periods of depression. In rabbits, cats, and rats, depression seemed to be the dominant symptom; while in frogs, depression alone was observed. "In dogs the first symptom shown is vomiting. This is soon followed by loss of muscular coordination. The hind legs are the first usually to be affected; then follow the fore leg muscles, and then those of the neck, jaw, and trunk." (Dresbach and Chandler, 1917.) The animal may recover after about one week,* or may die as a result of respiratory failure following one of the convulsions.

The symptoms of nitrobenzol poisoning may make their appearance during the course of the fumigation, immediately following it, or not until after several days. In dogs four days, and in chickens six days have elapsed between the time of exposure to the vapor and the onset of the symptoms.†

The action of nitrobenzol upon the tissues of animals poisoned by inhaling the vapor of this drug was studied by histological methods. It is interesting to observe that sections through the cerebellum of dogs thus poisoned (fixed in 10 per cent. formalin in saturated aqueous

* In most of the cases observed the recovery has been complete. Two cases are under observation, however, in which there appear to be permanent cerebellar lesions.

† The apparent degrees of resistance and of susceptibility to the action of nitrobenzol evidenced in animals, even in those of the same species, are extremely interesting. In one experiment three kittens, all of the same litter, were simultaneously fumigated at a temperature of 22° C. for a period of three hours. Two hours after the experiment was begun one of the kittens died. The other two were removed from the tank at the end of three hours and were apparently unharmed, except that digestive functions had been retarded, and never developed any symptoms of nitrobenzol poisoning afterwards. In another experiment, two chickens were fumigated at 23 C. for eight hours. One of them (♂), died shortly after being removed from the tank, while the other (♀), developed symptoms of poisoning only after about six days and the symptoms were then but slight.

solution of corrosive sublimate and stained by Nissl's method) show degenerative changes in Purkinje's cells, while no changes in any of the other cells of the entire central nervous system have been detected. A description of these changes with a discussion as to their significance will be presented in another paper.

The effects of nitrobenzol upon insects, especially fleas and biting lice, parasitic on the animals under observation were studied in connection with all of these experiments. The toxic action of this drug appears to run a more rapid course and to be more intense in the case of the insects observed than in the case of mammals and birds. Beginning shortly after the host was first exposed to the fumes, fleas could be detected crawling excitedly in and out through the hairs, migrating towards the anterior end of the host, apparently as the result of efforts to penetrate more deeply into the hair. In about one-half hour after an experiment was begun it was not an uncommon sight to see the nose of a dog or cat literally swarming with fleas. At 23° C. the activities of the fleas continue for about an hour, when the fleas become stupefied and are easily shaken off by the host. If removed from the vapor after an exposure of one and one-half hours, both the fleas and biting lice of dogs are apparently dead; but while a few of them do not recover, a large percentage of them recover within a short time; in fact, in all cases where conditions of exposure were milder than those corresponding to 26° C. for a period of six hours, a large percentage of both fleas and lice recovered. Likewise, a large percentage of the biting lice of poultry were found to recover after any exposure milder than that corresponding to 27° C. for a period of eight hours. On the other hand, in one experiment an exposure at 25° C. for a period of three hours was sufficient to cause the death of a dog, in another experiment an exposure of five hours at 22° C. caused the death of a dog, while in still another experiment a dog was exposed to the vapor at 20° C. for a period of ten hours and developed no symptoms of nitrobenzol poisoning at all.

In view of the fact that it is impossible to predict just what effect any given condition of exposure to the vapor of nitrobenzol will have on an animal, and the fact that it appears to be impossible to kill either fleas or biting lice by any condition of exposure under that corresponding to 26° C.* for six hours, it is clearly evident that this drug cannot be used with any degree of safety in the "fumigation of animals to destroy their external parasites."† However, since it seems hardly

* It has been suggested that while these insects recover after being removed from the vapor, they may eventually succumb to the action of the drug. Subsequent fumigations of the same animal have failed to prove this to be the case; however, much work is yet needed to be done on this score.

† The action of nitrobenzol upon internal parasites, particularly those inhabiting the blood, is at present being investigated.

probable that one hour's exposure to the vapor of nitrobenzol at temperatures between 20° C. and 25° C. will affect seriously any of the domesticated animals, while fleas and biting lice become stupefied after an hour's exposure at the same temperatures and are shaken off by the host in great quantities, it is quite possible that nitrobenzol fumigation may be used to good advantage in collecting specimens of external parasites. The following instances of its use for this purpose may be of interest:

A hen was fumigated at 25° C. for one hour. The hen was apparently unharmed and there were recovered from a sheet of paper previously placed in the bottom of the box more than two hundred specimens of external parasites. The fumigation was repeated on the following day and more than one hundred additional specimens were recovered, making a total of three hundred twenty-six specimens recovered. There were represented in this collection five genera and eight species as follows:

Specimen	Number taken
<i>Goniocotes hologaster</i>	63
<i>Goniocotes gigas</i>	4
<i>Lipeurus heterographus</i>	11
<i>Lipeurus variabilis</i>	2
<i>Menopon pallidum</i>	228
<i>Menopon biseriatum</i>	13
<i>Dermanyssus gallinae</i>	3
<i>Echidnophaga gallinaceus</i>	2
Total	326

In another experiment a hen was fumigated at 26° C. for one and one-half hours. The hen was unharmed and there were recovered more than five hundred specimens of biting lice. Among these were eleven specimens of *Goniocotes gigas*, a species which has been rarely collected in the vicinity of Ithaca.

A young kitten was combed with a fine-toothed comb for the purpose of collecting fleas, but not more than two fleas could be discovered. The kitten was then fumigated at 20° C. for a period of one and one-half hours. In about one-half hour after the experiment was begun the animal's nose was black with highly excited fleas. One-half hour later, the fleas were lying stupefied on the paper in the bottom of the tank. There were recovered eighty-nine specimens of *Ctenocephalus felis* and two specimens of *Trichodectes subrostratus*.

A dog was fumigated at 23° C. for about an hour, and there were recovered a number of *Ctenocephalus*, a great quantity of *Trichodectes latus*, and two specimens of *Haematopinus piliiferus*.

In the use of nitrobenzol fumigation for collecting purposes two advantages stand out: (1) Since the parasites become stupefied and are

readily shaken off by the host on a piece of white paper placed in the bottom of the box, great quantities of different species of external parasites may be collected with scarcely any trouble at all; (2) since the parasites are only stupefied and not dead, they may be revived and used for experimental purposes or killed by any method desired.

In the practical use of nitrobenzol fumigation in collecting external parasites the apparatus need not, of course, be so extensive as in experiments to determine the action of this drug on animals, and can be constructed at small cost by any laboratory. It should consist of a wooden or metal box provided with a tightly fitting lid. The size of the box should be determined by the size of the animal to be fumigated (for dogs, cats, and other small animals one of 20 cubic feet capacity will be sufficiently large). The box should be coated inside with paraffin to prevent rust in case metal is used, or to prevent absorption of moisture by the wood in case wood is used. It should have a false removable bottom of wire netting, and should contain a small wire cage which will serve to protect a container for nitrobenzol and the cloth (a triangular piece of cheese-cloth with a 6-inch base will serve) from which the nitrobenzol is evaporated.

The procedure is likewise simple. Preparatory to the fumigation of an animal, the bottom of the box should be covered with clean white paper and the false bottom placed upon this. Nitrobenzol (the commercial product will serve) is then introduced into the container and the box closed. After two or three hours have elapsed, the lid should be partly removed, the animal quickly introduced and the box closed again. After the animal has been exposed to the vapor for from one to one and one-half hours, the box should be opened and the insects, which will be found on the white paper, collected at once and placed in a warm, airy place, in order to insure the recovery of a maximum number.

Operators should be cautioned not to fumigate an animal at a temperature higher than 25° C. or for a longer period than one and one-half hours.

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A MORPHOLOGICAL STUDY OF BOTHRIOCEPHALID CESTODES FROM FISHES *

A. R. COOPER

In this study of North American cestodes of the order Pseudophyllidea the writer has used Lühe's (1902) revision rather than his later (1910) classification, since the family Caryophyllaeidae has not been considered. The order is represented in both marine and fresh-water hosts by a number of more or less isolated genera and species, three of the latter of which are new. It has been found necessary to expand the family Diphylobothriidae Lühe and to erect two new subfamilies, Haplobothriinae and Marsipometrinae, to accommodate the genera Haplobothrium Cooper and Marsipometra gen. nov., respectively.

DIPHYLLOBOTHRIDAE

The separation of this family from the Ptychobothriidae Lühe only on the basis of the presence or absence of a permanent sac-like enlargement of the uterus (the uterus-sac) and opercula on the eggs would probably now be considered as invalid, were there not other and perhaps more important characters for their differentiation. This is due to the presence of a uterus-sac in the genus Haplobothrium, as has already been emphasized by the writer (1914), and the form of the eggs in Marsipometra.

The only two genera of the subfamily Ligulinae Lühe, namely, *Ligula* Bloch and *Schistocephalus* Creplin, are present, each with the single species only that has been accepted by the European workers, particularly Lühe (1910). Both *L. intestinalis* Linnaeus and *Sch. solidus* (O. F. Mueller) are found in the advanced larval condition in the body-cavities of several small teleostean fishes, while the adults live in the intestines of piscivorous birds. Owing to the fact that there are several discrepancies among the existing descriptions of both of these forms, I found it necessary to make a detailed study of their morphology with the view to clearing up the situation.

As already pointed out (Cooper, 1914), the genus Haplobothrium occupies a unique position in the family. What was formerly considered to be the scolex (Fig. 3) is now known to be merely the foremost segment of the secondary strobila. The true scolex (Figs. 1 and 2) is cylindrical or club-shaped and, unlike any other Bothrio-

* Contributions from the Zoological Laboratory of the University of Illinois under the direction of Henry B. Ward, No. 95.

cephalid yet described, has as organs of attachment four eversible proboscides instead of two simple or modified bothria. It thus bears a remarkable resemblance to the scolex of the members of the order Trypanorhyncha, which is emphasized by the fact that each proboscis consists of an eversible portion, a muscular bulb for its activation, and a permanently protruded stump, quite comparable morphologically as well as physiologically with the three divisions of the trypanorhynchid proboscis. The manner of segmentation in this species is also peculiar. As shown in Figure 1, the larva or plerocercoid forms at its posterior end segments which by growth and subdivision in their anterior regions only become, after separation from the primary chain, the secondary strobilas already described (Cooper, 1914a). The genital organs resemble those of the subfamily Diphyllbothriinae Lühe in that they are simple in each proglottis while the openings are all surficial,* ventral and median. The vitelline follicles, as well as the testes, are situated, however, in the medullary parenchyma and within the nerve strands. Other characters which are important in the separation of this (Haplobothriinae) from the other subfamilies are the armament of the cirrus with minute spines and the division of the uterus into a much coiled proximal uterine duct and a large uterus-sac.

As has already been emphasized in the generic diagnosis (Cooper, 1914: 1-2) the nervous system consists of two chief strands situated in the medullary parenchyma outside of the vitelline follicles, uniting in the anterior end of the secondary strobila to form a nerve-ring, and eight collateral strands, four around each main tract, the latter in the jointed portion of the strobila only. In the true scolex, on the other hand, these main tracts are connected by an irregular transverse commissure (Fig. 4), situated among the proboscides, from which branches pass not only to the bulbs, but to the large ganglionic mass (Figs. 2 and 5) behind them. Four large tracts from the latter pass into the bases of the bulbs for the innervation of the retractor muscles of the eversible portions of the proboscides. In the secondary scolex the single large median and two smaller lateral excretory vessels all unite behind the nerve-ring to form a vesicle, but in the primary scolex they are connected not only by numerous large branches among the proboscis-bulbs, but by a single large median frontal loop.

The subfamily Cyathocephalinae Lühe is represented by both genera, *Cyathocephalus* Kessler and *Bothrimonus* Duvernoy. The species of the former, found only in *Coregonus clupeiformis* (Mitchill), the common whitefish, resembles the European *C. truncatus* (Pallas) in several points, but in others it is so radically different that it has been

* The word "surficial" has reference to the dorsal and ventral surfaces of the strobila.

considered as new and given the name *Cyathocephalus americanus* sp. nov. The chief nerve strands are connected anteriorly by a number of fine nerve commissures instead of a single one. The opening of the vagina is behind that of the uterus, while none of the genital apertures is surrounded by papillae or sphincter muscles. There is no enlargement of the vas deferens just before entering the cirrus-sac nor connective tissue sac surrounding the whole of the coiled duct as in *C. truncatus*. Furthermore, while the cirrus-sac is not provided with special dorsal retractor muscles, it is surrounded dorsally and laterally by a large mass of peculiar glandular pigmented cells (Fig. 9) the function of which has not yet been determined. The absence of a "connective tissue and muscular sac" surrounding the beginning of the vagina and any such "shell-gland" as described for the European form are also of great importance from a morphological as well as a systematic standpoint.

The generic name *Bothrimonus* Duvernoy has been taken to include both *Bothrimonus* and *Diplocotyle* Krabbe, as contended by Schneider (1902). Of this genus there is present only one species, *B. intermedius* sp. nov., which, while resembling *B. nylandicus* Schneider (1902) in particular, differs from it rather in an aggregate of details than in a limited number of special diagnostic value. The nervous system is, however, radically different in that just behind the scolex each chief strand divides into two sagittally directed branches which are united frontally by a diffuse and not compact commissure.

I have given the name *Marsipometra* gen. nov. to *Dibothrium hastatum*, briefly described by Linton in 1897, owing to the fact that it cannot be accommodated in any of the existing genera, altho it has several features in common with some of those belonging to the subfamily *Triaenophorinae* Lühe. The sagittate scolex (Fig. 10), the proglottides, and the segmentation itself are all quite distinct and regular in their nature. Nor is this confined to the external features, for the arrangement of the internal organ-systems is also peculiarly diagrammatic (Fig. 11). The opening of the cirrus and vagina at the bottom of a comparatively capacious genital cloaca (Fig. 13) is marginal and irregularly alternating, while the uterine aperture is surficial, ventral, and on the same level with the genital atrium or very slightly behind it. A conspicuous hermaphroditic duct and a cloacal sphincter are also present. The testes are situated in the medulla between the nerve strands, which are far towards the margins and dorsal to the cirrus-sac and vagina, and are arranged in two lateral fields united ahead of and behind the uterus-sac and central genital ducts. There is no vesicula seminalis immediately proximal to the cirrus-sac, but the receptaculum seminis is comparatively large, rather long, and sharply

separated from the continuation of the vagina, the spermiduct. The ovary is comparable to that of some of the members of the Triaenophorinae in that it is not exactly in the median line, but slightly approaching the margin bearing the genital cloaca. It is also reniform, with tubulolobular wings and a thick, ventrally situated isthmus (Fig. 11). The shell-gland, ahead of the ovary, is likewise not exactly in the median line, but towards the genital cloaca. The vitelline follicles, as shown in Figure 11, are arranged among the body muscles, not in two lateral fields, but continuous from side to side in the anterior and posterior regions of the proglottis. Like the testes they are not continuous from proglottis to proglottis. The uterus-sac is pouched (whence the generic name) and occupies the whole of the medulla dorsoventrally, but not transversely in gravid joints. As in the genus *Bothriocephalus*, it is developed by the enlargement inwardly of that portion of the duct passing thru the cortical parenchyma. The uterus-opening is towards the margin bearing the genital cloaca, especially in the younger segments; in gravid proglottides it is naturally more median, but never exactly in the median line. Perhaps the most outstanding feature of *Marsipometra hastata* is the fact that the eggs are not provided with opercula, in which particular it is isolated from all other members of the family Diphylobothriidae. Finally, as regards its systematic position, my study of the anatomy of this species leads me to conclude that not only must it be accommodated in a few genus, but also in a new subfamily the name for which will then be Marsipometrinae subfam. nov.

In the material studied the subfamily Triaenophorinae Lühe was represented by *Triaenophorus* Rudolphi and *Fistulicola* Lühe. Altho all of the specimens of the former were larval, two forms from several host species were recognized as being probably the same as the European *T. nodulosus* (Pallas) and *T. robustus* Olsson. *Fistulicola* is, on the other hand, represented by the single species *F. plicatus* (Rud.) from *Xiphias gladius* L., the swordfish.

PTYCHOBOTHRIDAE

Lühe's use of the distinction between the two main divisions of the uterus, the uterine duct and the uterus-sac or uterus proper, in the separation of this family from the Diphylobothriidae, is justified by my study of the developmental relationships of these parts with each other in both *Bothriocephalus* s.str. Lühe and *Clestobothrium* Lühe. In the species of these two genera the common rudiment of the uterus separates into its two constituents soon after it is formed, which has also been shown (Cooper, 1914 and 1914a) to obtain in the case of *Haplobothrium globuliforme*. Furthermore, in most of the species of

the former genera there is to be seen a method of segmentation of the strobila, which, so far as I am aware, has not yet been described. It consists of a gradual and more or less regular subdivision of a primary segment, which arises from the base of the scolex, into secondary, tertiary, and quaternary subsegments, and even those of the fifth and sixth orders; or into two, four, eight, sixteen, and thirty-two (or more), respectively. Usually, however, the formation of these subsegments does not proceed with the same degree of regularity in all parts of the primary segment, as shown in Figure 18, where the sizes of the dots at the side indicate the values of the subdivisions; there is a sort of dominance of the anterior over the posterior region. This also applies, as can be seen, to the major segments as well, and is on the whole quite comparable to the dominance observed in the experimental regeneration of portions of various planarian worms.

Both of the two well known European species of *Bothriocephalus*, namely, *B. scorpii* (O. F. Mueller) (= *B. punctatus* Rud., = *B. bipunctatus* Lühe) and *B. claviceps* (Goeze) are recognized and accepted by the writer as American species also, while *Dibothrium manubriforme* Linton and *D. occidentale* Linton are redescribed and placed in the genus *Bothriocephalus*. *D. laciniatum* Linton and *Bothriocephalus histiophori* Shipley are deleted owing to the fact that they are both considered to be identical with *B. manubriforme*. On the other hand, a fresh-water form, found chiefly in *Stizostedion vitreum* (Mitchill), the wall-eyed pike, is described as new under the name of *Bothriocephalus cuspidatus* sp. nov. It is a medium-sized cestode, up to 180 mm. in length by 2.75 mm. in breadth. The comparatively large scolex (Figs. 14 and 15) is provided with a very prominent terminal disc, deeply notched superficially, and long narrow bothria, quite deep posteriorly. The first segments, while subcuneate in outline and with prominent posterior borders, are almost circular in transection; the middle gradually broaden until they become much broader than long; and the posterior are two to four and a half times wider than long. The genital cloaca is deep and funnel-shaped, and into it the vagina opens close behind the cirrus, the hermaphroditic duct being obscure. The testes on each side of the median genital complex are separated into two unequal fields by the nerve strand (Fig. 16). The cirrus-sac (Fig. 17) is quite large and prominent, being as much as 0.25 mm. in length (depth) and 0.20 mm. in diameter; as shown in Figure 16, it is not exactly median in position. While the ovary is a rather compact organ, the vitelline follicles occupy almost the whole of the cortex, are very numerous (800 to 1000 per proglottis), and are strongly united dorsally and ventrally as well as laterally. The uterine duct is confined to one side of the median line and constantly opposes the

cirrus-sac or coils of the vas deferens, depending on the degree of maturity of the genitalia, both alternating irregularly from side to side. The spherical uterus-sac, on the other hand, occupies when gravid one-third of the transverse diameter of the segment and has its opening in the median line very close to the anterior edge of the latter.

Cleistobothrium crassiceps (Rud.), the type and only species of the genus, occurs only in *Merluccius bilinearis* (Mitchill), the silver hake or whiting of the Atlantic coast. Its anatomy has been thoroughly studied by the writer and the somewhat meager descriptions in the European literature greatly augmented, some errors being at the same time corrected.

The other subfamily of this family, the Amphicytylinae Lühe, is represented by the genus *Abothrium* van Beneden only. Of this genus only *A. rugosum* (Batsch) and *A. crassum* (Bloch) are found in general in marine Gadidae and Salmonidae, respectively. Specimens from *Lota maculosa* LeSueur were considered to belong to the latter species, altho in Europe *Lota* has been said to harbor the former (vide Lühe, 1910). As a matter of fact, *A. rugosum* presents not a few difficulties as regards its specific identity in *Lota* in particular, which was keenly felt by the writer in the absence of European material for comparison.

Apart from the species dealt with here the following have also been reported from fishes in America: *Dibothrium* (*Anchistrocephalus*) *microcephalus* (Rud.), *D. aleuteræ* Linton, *D. tortum* Linton, *D. cynoscioni* [Linton] Ariola, *D. cordiceps* Leidy, and *D. speciosus* Leidy; but since adult material of none of these was available for study, they must remain for the time being at least as *species inquirendæ*. The same may also be said in a certain sense of *Bothrimonus sturionis* Duvernoy 1842, which needs to be reinvestigated from the standpoint of the differentiation of the species of and of the genera *Bothrimonus* and *Diplocotyle* (vide supra).

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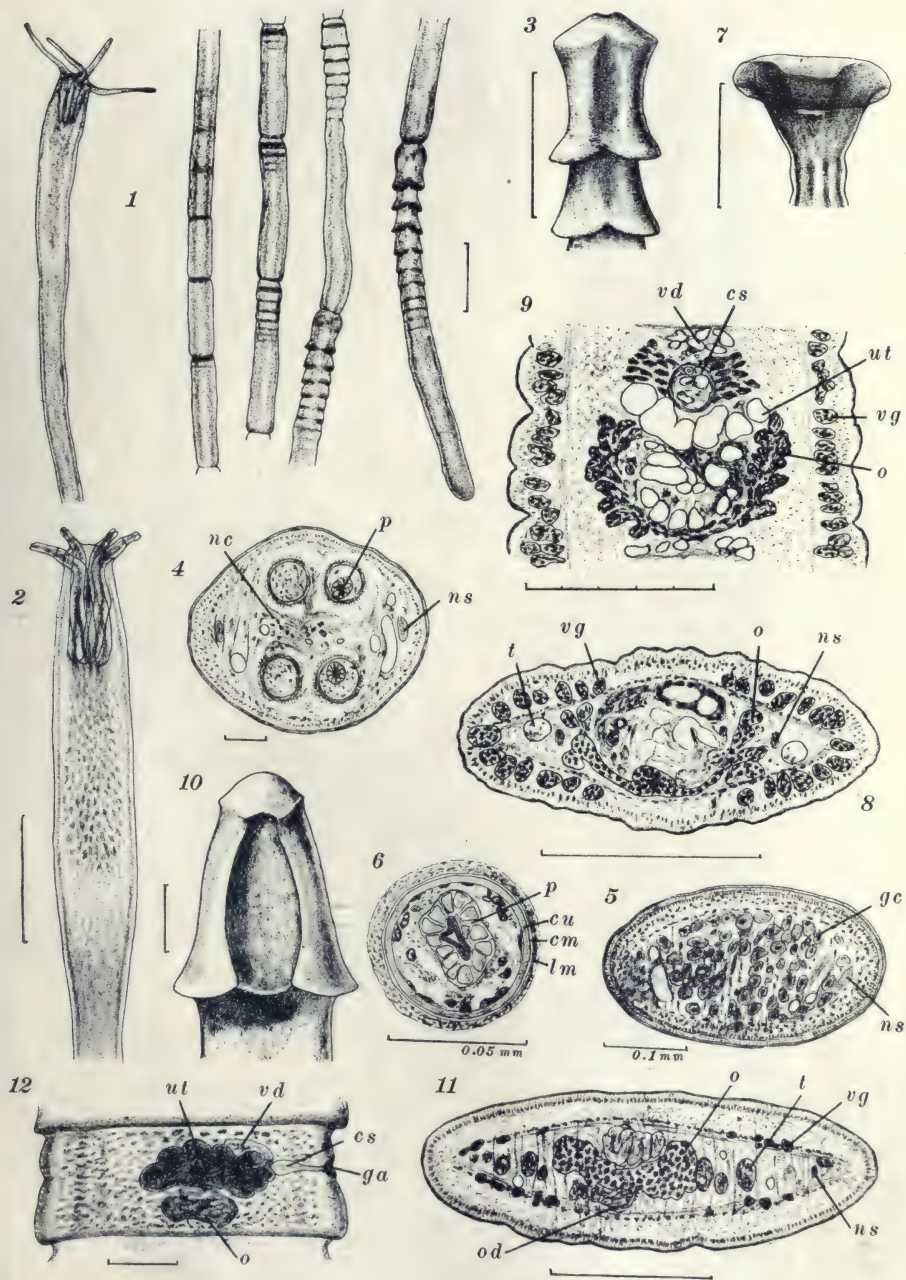


PLATE 1



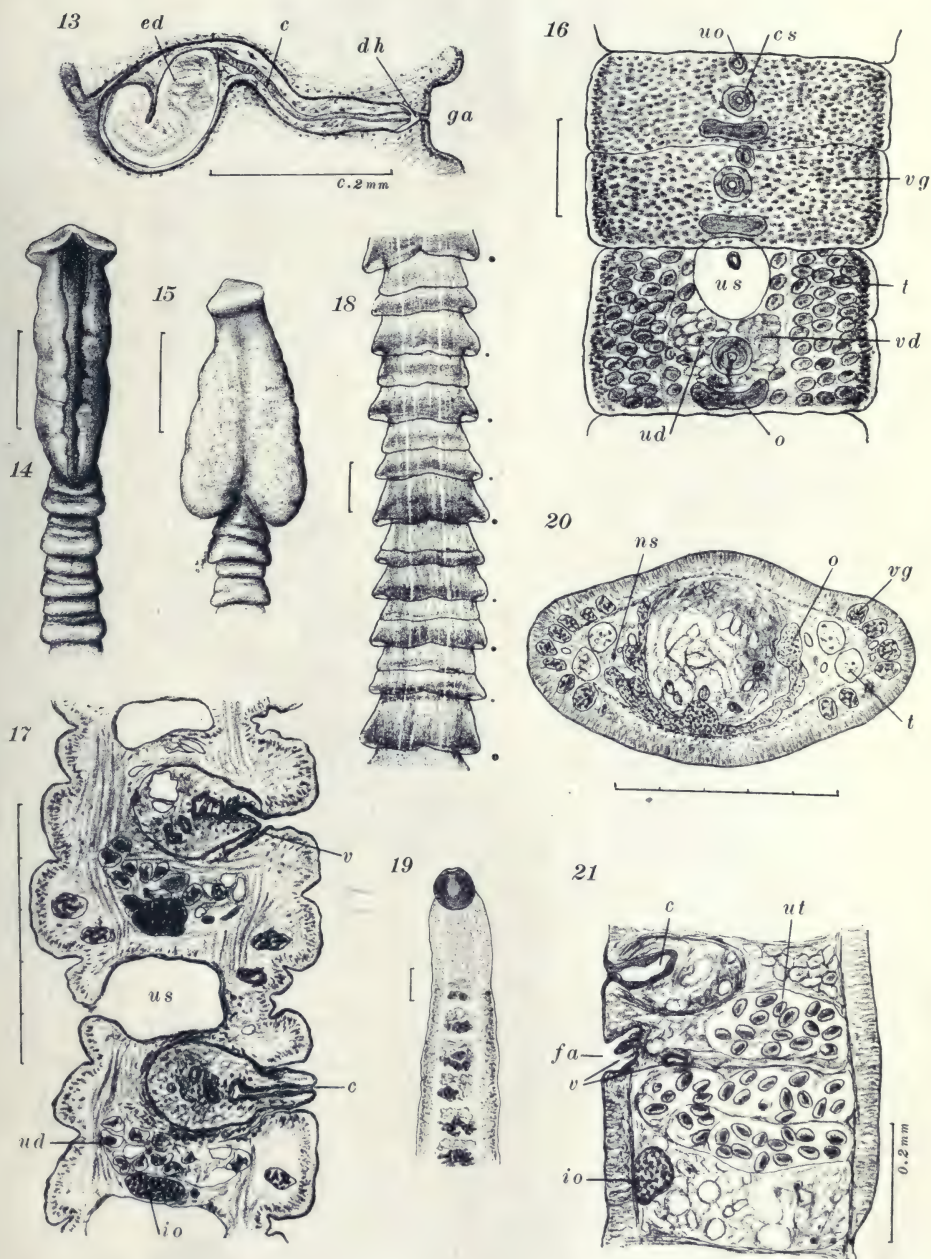


PLATE II



EXPLANATION OF FIGURES

All drawings were made with the aid of the camera lucida. Unless otherwise labeled, the lines indicating the magnifications are 0.5 mm. in length.

<i>c</i> cirrus	<i>ns</i> nerve strand
<i>cm</i> circular muscles	<i>o</i> ovary
<i>cs</i> cirrus-sac	<i>od</i> oviduct
<i>cu</i> cuticula	<i>p</i> proboscis
<i>dh</i> hermaphroditic duct	<i>t</i> testis
<i>ed</i> ejaculatory duct	<i>ud</i> uterine duct
<i>fa</i> female atrium	<i>uo</i> uterus opening
<i>ga</i> genital atrium	<i>us</i> uterus-sac
<i>gc</i> ganglion cells	<i>ut</i> uterus
<i>io</i> isthmus of ovary	<i>v</i> vagina
<i>lm</i> longitudinal muscles	<i>vd</i> vas deferens
<i>nc</i> nerve commissure	<i>vg</i> vitelline glands

PLATE I.

Fig. 1.—*Haplobothrium globuliforme*, primary strobila, showing formation of secondary strobilas, toto preparation.

Fig. 2.—*Haplobothrium globuliforme*, scolex, toto.

Fig. 3.—*Haplobothrium globuliforme*, secondary scolex, surficial view.

Fig. 4.—*Haplobothrium globuliforme*, transection through scolex.

Fig. 5.—*Haplobothrium globuliforme*, transection through the ganglionic mass.

Fig. 6.—*Haplobothrium globuliforme*, transection through single proboscis bulb.

Fig. 7.—*Cyathocephalus americanus*, scolex, toto.

Fig. 8.—*Cyathocephalus americanus*, transection through ovarian isthmus.

Fig. 9.—*Cyathocephalus americanus*, frontal section of ripe proglottis.

Fig. 10.—*Marsipometra hastata*, scolex, surficial view.

Fig. 11.—*Marsipometra hastata*, transection through ovarian isthmus.

Fig. 12.—*Marsipometra hastata*, toto of ripe proglottis.

PLATE II

Fig. 13.—*Marsipometra hastata*, cirrus-sac from a transection.

Fig. 14.—*Bothriocephalus cuspidatus*, scolex, surficial view.

Fig. 15.—*Bothriocephalus cuspidatus*, same, lateral view.

Fig. 16.—*Bothriocephalus cuspidatus*, toto of ripe proglottides, posterior in deeper optical section.

Fig. 17.—*Bothriocephalus cuspidatus*, median sagittal section, composite.

Fig. 18.—*Bothriocephalus manubriiformis*, an anterior primary segment; the sizes of the dots at the side indicate the values of the subdivisions.

Fig. 19.—*Bothrimonus intermedius*, toto of anterior end, showing genitalia.

Fig. 20.—*Bothrimonus intermedius*, transection through ovarian isthmus.

Fig. 21.—*Bothrimonus intermedius*, median sagittal section.

NOTES ON KNOWN GREGARINES*

MINNIE WATSON KAMM

The following notes relate to the systematic position of two known species of gregarines. In the one is described and named a species seen but not named by Leidy; in the other is substantiated the determination made by Crawley for a species which he named from two of the three essential characters.

LEIDYANA LEIDYI Kamm *nov. spec.*

[Figures 1, 2, and 3]

Host: *Nyctobates pennsylvanica* deGeer

Habitat: Intestine

Location: Urbana, Illinois, December, 1916

The sporont of this species (Fig. 1) is long and slender, tapering at both ends. The protomerite is only half as wide as the deutomerite at the widest portion; it is slightly constricted at the septum and terminates in a blunt point. The deutomerite is widest in the shoulder region, i. e., a short distance below the septum, and tapers from thence to a long cone blunt at its extremity.

The epimerite (Fig. 3) is a spherical, sessile knob placed at the apex of the protomerite of the cephalont.

The nucleus, obscured in life by dense protoplasm, is spherical and small, situated generally above the median portion of the deutomerite; it contains from one to five large, irregular, deeply-staining karyosomes.

The endocyte of the deutomerite is dense, staining dark and homogeneous, while that of the protomerite is less compact and consists of much larger protoplasmic granules. In transmitted light, the protomerite appears deep tan in color, while the deutomerite is black in the upper portion and gray-black in the lower where the protoplasm is less dense. The epicyte is clear, much thicker in the protomerite, especially at the sides of the septum and in the apical region.

It is apparent that more than one species of gregarine parasitizes this host-beetle. Leidy (1889) describes and illustrates *Asterophora*

* Contributions from the Zoological Laboratory of the University of Illinois under the direction of Henry B. Ward, No. 96.

philica from this host, an attenuated species having for an epimerite "a horizontal circular disc with a round, milled border." The species attains a length of two millimeters, with a ratio of length protomerite to length deutomerite of one to fifteen, and that of width protomerite to width deutomerite of one to one and three-tenths. From an unpublished manuscript of Leidy on gregarines, Crawley (1903a) copied three figures (Pl. III, Figs. 31, 32, and 33), supposedly of the same species, *A. philica*, and taken from the same host-beetle as above. While doubting the authenticity of their relative positions as assigned by Leidy, Crawley does not attempt to further classify them because of the slender evidence, calling all three *A. philica* as done by Leidy.

In my thesis (Watson, 1916) the fact was mentioned (p. 144) that the first of the figures represents undoubtedly the species *A. philica* originally seen as described by Leidy in 1889, for the shape, propor-

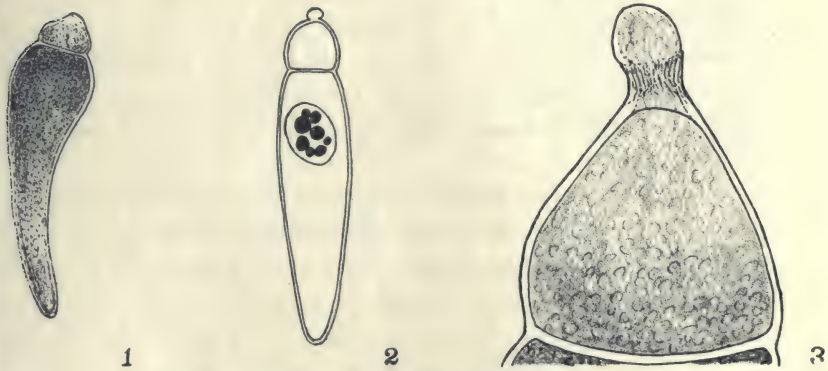


Fig. 1.—Sporont of *Leidyana leidy* nov. spec.

Fig. 2.—Sporont copied from Crawley (1903a, Pl. III, Fig. 32).

Fig. 3.—Protomerite with epimerite, oil immersion.

tions, and furrowed-disc character of the epimerite agree in the two. It was further mentioned that the second drawing in the Crawley paper (reproduced in this paper as Figure 2) "may or may not be a cephalont of the same species." But, from the data presented above, it is now evident that this cephalont, which has a sessile knobbed epimerite, represents not the same species as the first drawing, but another, the chief differentiating character being the epimerite. In *Asterophora philica*, the epimerite is a horizontal, peripherally milled disc, while, from the abundance of epimerited specimens which I have seen, it is a simple spherical knob with no trace of corrugations even under oil immersion. Therefore, the second of the drawings in the Crawley paper and the specimens I have seen correspond and

represent a species which is not *Asterophora philica* Leidy. Below is a table of the contrasting features of the two species:

	<i>A. philica</i> (Leidy 1889)	<i>Leidyana leidy</i> n.sp.
Sporont, maximum length recorded	2000 μ	300 μ as recorded by Crawley from Leidy's Mss. 550 μ from my specimens
Maximum width....	150 μ	180 μ
Ratio LP:TL, maximum	1:15	1:11
Ratio WP:WD, maximum	1:1.3	1:2
Epimerite	A flat horizontal disc with milled border	A smooth, spherical knob

While it is true a single species may undergo decided changes in different environment and even within the same host, yet certain characters are fixed and are used in differentiating species; one of these is the character of the epimerite. It is because of this deviation of the epimerite from the named species that the writer assigns to the specimens seen by Leidy and illustrated by Crawley and to those taken recently a new name.

A new genus has been named to include species living solitary in the sporont stage (until the actual time of cyst-formation), having spherical cysts, numerous spore-ducts, dolioform spores, and a simple globular sessile epimerite, as *Leidyana* (Watson 1915), differing from the genus *Gregarina* only in the fact that the animals are solitary instead of biassociative during the sporont stage. In the absence of any data concerning spores and cysts, and because these two genera alone among known gregarines possess simple knobbed epimerites, the present species is placed in that genus as a new species, *Leidyana leidy*.

A table of measurements of the new specimens in microns follows:

Length protomerite (without epimerite, if present)	20	23	50	50
Length deutomerite	150	187	400	500
Length epimerite (if present)		10		
Width epimerite		10		
Width protomerite	24	40	120	70
Width deutomerite (maximum)	140	75	180	140
Total length sporont	170	220	450	550
Ratio LP:TL	1:8.5	1:9.6	1:9	1:11
Ratio WP:WD	1:1.6	1:1.9	1:1.6	1:2
Ratio LP:TL (Crawley's copy of Leidy's figure of cephalont)	1:6			
Ratio WP:WD (Leidy's figure)	1:1.2			

ACTINOCEPHALUS HARPALI (Crawley)

Gregarina harpali, Crawley, 1903a: 49-50*Actinocephalus harpali*, Crawley, 1903b: 637-38Host: *Harpalus caliginosus* Fabr. (Carabidae).

Determined by Adam Boving

Habitat: Intestine

Location: Atlanta, Georgia, July, 1916

This species, already described, is mentioned here because the epimerite has not heretofore been seen. Crawley has adequately described the sporonts, cysts, and spores, the only stage not seen being that of the cephalont.

The epimerite of the cephalont consists of a small flat disc at the apex of the protomerite and surrounded by a corona of six to nine short, broad, digitiform processes, conforming with that of the type species of the genus in which the species was placed.

This addition to Crawley's description confirms his disposition of the species and completes all the specific characters by which a species is recognized.

That the distribution of the species is rather extended is seen by the fact that the two localities from which it has been taken are Pennsylvania and Georgia.

Additional data is given as to measurements, since the original description mentions only the length as from 225μ to 700μ . Dimensions are in microns.

Length epimerite, if present.....	20	20	40	
Width epimerite	60	50	50	
Length protomerite, without epimerite	150	170	170	210
Length deutomerite	610	730	880	890
Total length sporont	760	900	1050	1100
Width protomerite	210	170	200	180
Width deutomerite	250	250	250	200
Ratio LP:TL	1:5	1:5.3	1:6	1:5.2
Ratio WP:WD	1:1.2	1:1.4	1:1.2	1:1.1

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FIRST CASE OF LEISHMANIOSIS CUTANEA IN VENEZUELA*

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AND

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Credit must be accorded to Lindenberg (1909) for having identified the Bauru ulcer of the state of Sao Paulo, Brazil, with the Biskra Button. In substance, the cause of those tegumentary ulcerations is a protozoon of circular or oval shape, of 2 to 4 μ in length and 1 to 2 μ in width, classified by Vianna (1913) under the denomination of *L. braziliensis*, the specific agent of the leishmaniosis cutanea, a disease known in some parts of Venezuela by the vulgar name of festering wound.

L. braziliensis in preparations colored with Giemsa's stain presents an oval-shaped nucleus of violet tint situated near the anterior extremity; its protoplasm is but slightly affected by the coloring matter and is somewhat bluish.

The kintonucleus or blepharoplast is located exactly in the line of the lesser diameter of the protozoon. The fundamental character of the Leishmania is the presence in the interior of the protoplasm of a stained band of pale red, situated perpendicularly to the kintonucleus, which is called *rizoplate*.

The disease has been described in other states of Brazil by Carini (1909), Piraja da Silva (1912), and Matta (1910); in Surinam, by Flu (1911); in Peru by Escomel (1911) and Gastiaburu (1913); in Paraguay by Migone (1913); in Bolivia by Sagarnaga (1912); in Panama by Darling (1911), and in French Guiana by Nattan-Larrier and Heckenrath (1909).

The patient who was the subject of the present discussion, as well as the related microscopic preparations, were submitted to the National Academy of Medicine and studied in our laboratory by Drs. Gorgas, Guiteras, and Carter, members of the Yellow Fever Commission of the Rockefeller Institute, who confirmed our diagnosis.

* Paper read at the Second Venezuelan Congress of Medicine, assembled at Maracaibo, 1917. (Contribution from the Laboratory of Dr. Juan Iturbe, Caracas, Venezuela.)

X. X. arrived at our clinic from San Fernando de Apure, where he resides and is engaged in the cattle industry. He is a peasant cattleman of our friend C. E.

He states that two years previously he suffered in both legs various pruriginous acne, hard, violet colored, resisting all medication. These tumors increased in extent until they ulcerated. Some of them healed spontaneously, while others remained in the same state; the ulceration was characterized by hard edges, a cover of black crust, and bad odor.

In the month of August of last year he decided to consult us, having had no improvement from any of the treatments to which he had been submitted.



As may be seen in the photoplate which accompanies this note, the lesions of the skin are localized in both legs. In the right forearm and the knee of the same side movable nodules may be readily observed situated in the sub-dermic region. During the course of his illness X. X. does not remember to have suffered from fever.

The examination of the blood gave the following result:

Red corpuscles.....	4,800,000
White corpuscles.....	10,000
Hemoglobin	73:100

Leukocytal formula

Polynuclear eosinophiles.....	11 %
Polynuclear basophiles.....	33.5%
Mononuclear	2.9%
Large lymphocytes.....	18 %
Small lymphocytes.....	32 %
Transitional forms.....	2 %

The Wassermann reaction was — — —. The preparations effected with the serosity and the blood of the lesion, previously scraped, colored with Giemsa's stain, showed the presence of a great quantity of *L. braziliensis*.

This case was submitted to the emetic treatment, following the methods of Vianna (1912 and 1914), Carini (1914), and Utra Silva (1915). One month after treatment, the cure was definite.

We employed the emetic of Baiss Brothers in an aqueous solution of 1 per cent. Sterilization is done by filtering cold through a Berkefeld filter. Every two days there will be intravenous injections of 5 c.c. of the solution referred to, until cure is complete. Care should be taken to inject the liquid as slowly as possible, in order to avoid the fits of coughing and muscular pains which are apt to result when the emetic solution is introduced rapidly into the vein.

Lindenberg (1913) has employed also in this disease trixidine (oleaginous emulsion of trioxide of antimony), a substance recommended by Kolle (1913) for the treatment of trypanosomiasis. This has given excellent results.

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NOTE.—Since this paper was put in shape for publication several other cases of Leishmaniosis have been observed in Venezuela. One case treated by intravenous injections of emetin has been sent to me by one of my colleagues in the interior of the country; it has already had a duration of four years.

BOOK REVIEWS

THE KITASATO ARCHIVES OF EXPERIMENTAL MEDICINE. Edited by S. Kitasato, associate editor, K. Shiga. Volume I, number 1; April, 1917. Tokio, Nippon.

The appearance of a new journal in this field would call for comment even if it were not sponsored by the distinguished names of the editor and his associate. In the introduction Professor Kitasato writes of the establishment in Tokio in 1892 of the institute now under government control which bears his name and has made itself a force in the Orient by its work. He refers also to the progress of the Japanese nation in the science of medicine and emphasizes justly the international character of scientific studies, adding in closing the introduction:

"I have been aware that foreigners have wanted to know what the Nippon medical fraternity has been doing in the way of scientific investigation but linguistic difficulties have thus far prevented them from doing so. We hope by the publication of these Archives in English, French and German, to make good this deficiency and to introduce to a wider circle the results of our efforts, and thus bring Nippon medicine to the attention of the world."

The new periodical deserves especial mention in these pages because of the contents of this first number. The introductory article On the Life Cycle of the "Akamushi," Carrier of Nippon River-fever, by Inaka, deals with the structure and development of the red bug or mite by which the disease is transmitted. In his work the author comes to the conclusion that the mite represents a form different from any known adult, and to it Nagayo has given the name *Leptotrombidium*; the species is then *Leptotrombidium akamushi* (Brumpt).

The third article treats of the ictero-hemorrhagic spirochetosis (Weil's disease) for which Inada in 1915 found the probable cause in *Spirochaeta ictero-haemorrhagiae*. A considerable part of the article is devoted to the organism and to the rôle of rats in its transmission. The last article discusses a new stain for the coloration of protozoans and of blood corpuscles.

The Archives are well printed and splendidly illustrated. The eight plates challenge comparison with any made in other countries.

It is noteworthy that so large a part of this first number is taken up by studies in medical zoology which are marked by their breadth and scientific character. THE JOURNAL extends its congratulations to the editors of the new Archives with best wishes for its continued success as a worthy representative of medical research in a great nation.

Kobayashi has published in the *Mitteilungen der medizinischen Fachschule zu Keijo* an extended study on the life-history and morphology of the liver distome (*Clonorchis sinensis*). In all twelve fishes have been found to harbor the encysted distome and are the source of human infection. From 23 to 26 days are required for the attainment of complete maturity in the final host; during this period spines appear and then disappear, a fact which has led to a difference in the descriptions of the worm given by various authors. All the Japanese liver distomes are really a single species and not as claimed by Looss two forms, one large and one small with differences in structure and range. The work contains a mass of detail to support these and other findings, and is illustrated by five fine plates.

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HOMOLOGIES OF THE EXCRETORY SYSTEM OF THE FORKED-TAILED CERCARIAE

A PRELIMINARY REPORT *

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Very little is known of the homologies of the protonephridial system in the various subdivisions of the Trematoda. Most of the anatomical studies on this group have been made from toto mounts and serial sections of preserved material, in which locating the flame cells and tracing the finer branches of the excretory system is practically impossible. My work has convinced me that a more complete knowledge of this system will do much to clear up relationships and to establish natural families. Also an increased knowledge of the excretory systems of little known types of cercariae will be of great help in solving life-histories by suggesting the groups of adults to which such forms belong. In certain cases the close relationship of two cercariae may be shown by comparisons of their excretory systems; when on account of differing degrees of development of adaptive larval characters they superficially appear to be very different.

The work on larval trematodes of which this paper forms the first published part was carried on at the University of Michigan Biological Station at Douglas Lake, Michigan, during the summers of 1914, 1915, and 1916. The region around Douglas Lake is very favorable for work on these forms since an abundance of material of a large variety of trematodes is readily available. I wish to express my appreciation to the University of Michigan Biological Station for facilities provided for carrying on this work. Thanks are due Mr. H. B. Baker for the identification of the molluscan hosts.

The best results in tracing the excretory systems of larval trematodes can be obtained from high power studies on living animals. To make such work effective it is important to have an abundance of material. By using thin cover glasses and slowly removing the water from the preparation even cercariae so small that they are almost invisible to

* Publication from the University of Michigan Biological Station.

the naked eye can be slowed down and flattened so that they can be studied under the oil immersion. Just before a cercaria goes to pieces from drying and flattening the movements of the flame cells are accentuated and the smaller tubules distended so that they become clearly visible. The use of large numbers of preparations, which is easily possible on account of the great numbers of cercariae in one infected snail, will gradually reveal the number and position of the flame cells and the pattern of the tubules. Larger agamodistomes* and adults can be mounted in a ring of vaselin, somewhat compressed and studied living for several hours or even a whole day. Fully developed cercariae should be mounted for this kind of study in pond water and agamodistomes and adults in normal saline. Aside from the excretory system much valuable data on the methods of locomotion, movements, and changes in shape, can be gained only from the study of the living animal. In fact in most cercariae it is possible to work out the structure much more completely in living than in preserved material. Looss (1894: 3) very strongly advocates the use of living animals in the study of trematodes and much of his most important morphological work on this group was accomplished by this method. La Rue (1917: 5) in a recent paper in which he describes two agamodistomes from snakes makes an appeal for the use of better and more varied staining methods in the study of parasitic worms. I would add to this a strong plea for more study of the living animals especially when dealing with cercariae, agamodistomes, or small adult trematodes. Much unsatisfactory work and many errors can be avoided by the adoption of these two improvements in methods of work.

The present paper consists of a comparison of the excretory systems of five forked-tailed cercariae, of which two, *Cercaria douthitti* Cort and the cercaria of *Schistosoma japonicum*, are already known and the others are new species. *Cercaria douthitti* was described from *Lymnaea reflexa* Say from the vicinity of Chicago, Illinois (Cort, 1915: 49), but at that time only a part of the excretory system was worked out. The cercaria of *Schistosoma japonicum* has been described by several Japanese workers but its anatomy has not been adequately worked out. In this connection I describe only the excretory system of this cercaria since a detailed account of its anatomy will appear in the near future in the Zoological Series of the University of California Publications. My material of the cercaria of *Schistosoma japonicum* was obtained from infected snails sent to Professor C. A. Kofoed from Japan by Professor A. Fujinami of the University of Kyoto. To both of these gentlemen thanks are due for making possible my studies on this

* The term agamodistome (from Agamodistomum Stossich 1892) I accept for larval trematodes when known only in the encysted or "resting stage."

species. The three new cercariae are from the Douglas Lake region. One of these, a very large form from *Planorbis trivolvis* Say, I name *Cercaria elephantis* from its large size and from the fact that its body when seen from side view after the loss of its tail resembles an elephant's head. The second of the new forms was found in *Lymnaea emarginata angulata* Sowerby and is given the name *Cercaria emarginatae* and the third from *Physa ancillaria* Say is called *Cercaria douglasi* from Douglas Lake. These three species and several others of the forked-tailed cercariae not included here will be described in a later publication. Recently the forked-tailed cercariae have been brought into prominence by the work of Leiper and others on the life-histories of the human schistosomes, so that it becomes very important that this group should be carefully worked up.

There has been a tendency since the discovery of the life-histories of the human schistosomes to relate all forked-tailed cercariae to this group without making careful analysis. Faust (1917:119) in a description of two forked-tailed cercariae makes the following statement: "This group of larval trematodes (the forked-tailed cercariae) is characterized by a forked tail and as far as the writer knows, the absence of a true pharynx. However, glands of the pharyngeal region may lead one to consider the mass a pharynx, which is evidently the error Looss (1896) made in the study of *Cercaria vivax* Sons." Looss' figures of both immature and mature specimens of *Cercaria vivax* (Looss, 1896, pl. xv, figs. 174-176) show the structure of the pharynx so clearly outlined that it seems impossible he could have erred in this particular. Now my discovery of other forked-tailed forms with pharynges establishes this as a definite group of the forked-tailed cercariae and shows that within this group, which my observations on the excretory system indicate to be a natural one, there are represented two different families.

Cercaria douthitti Cort 1914

In the summers of 1915 and 1916 *Cercaria douthitti* was found in the region of Douglas Lake, Michigan, in *Lymnaea stagnalis appressa* Say and *Lymnaea stagnalis perampla* Walker. Besides the completion of the study of the excretory system there are several points which should be added to my original description of this species. Careful studies of living specimens show the outlines of the esophagus and the intestinal ceca as far as the acetabulum. Whether they end here or are merely masked in the posterior region by the mass of glands could not certainly be determined. It was also found that the whole surface of the body and tail is evenly covered with very minute spines which are visible only with the highest power of the microscope. Also the number of cephalic glands is ten instead of eight.

The excretory bladder of *Cercaria douthitti* (Fig. 1, *b*, *tb*) extends the length of the tail and divides to open at the tips of its bifurcations. At the base of the tail where the bladder passes from the body into the tail the fused nature is indicated by a small island (*i*). The part of the bladder in the body proper (*b*) which will remain after the loss of the tail as the bladder of the adult is V-shaped. The sides of the V extend up to the anterior margin of the acetabulum and turn backward to points (*p*) near the sides of the body at the level of the posterior margin of the acetabulum, where they receive the anterior and posterior collecting tubes (*act*, *pct*). A short region of the bladder on each side near the points where the collecting tubes enter is ciliated. The anterior collecting tube receives capillaries from three flame cells, one at the level of the posterior margin of the oral sucker, the second at the level of the eye-spots, and the third at about the level of the anterior margin of the acetabulum (*ff*). The posterior collecting tube on each side also receives three capillaries, two from flame cells in the postacetabular region and the third from a very large flame cell in the anterior region of the tail. The character of these flame cells is shown in Figure 2*D*. This is the type of flame cell found in all of the forked-tailed cercariae studied. The flame cells and tubules of the body region are limited to narrow areas at the sides and do not invade the central core of the body which is crowded full of unicellular glands (Cort, 1915, Figs. 55, 56).

The *Cercaria* of *Schistosoma japonicum*

The excretory system of the cercaria of *Schistosoma japonicum* is shown in Figure 2*A*. The bladder corresponds to that of *Cercaria douthitti* except that it extends further in front of the acetabulum. The anterior and posterior collecting tubes show distinctly greater caliber than the capillaries. The anterior collecting tube receives two capillaries on each side from flame cells in the preacetabular region and the corresponding posterior collecting tube also receives two capillaries, one from a flame cell near the posterior end of the body, and the other from one in the anterior region of the tail. In all there are six flame cells in the body arranged along the sides and two in the anterior region of the tail. A comparison of this type of system with that of *Cercaria douthitti* shows that they are homologous except for the number of flame cells.

Cercaria elephantis nov. spec.

Forked-tailed cercaria without pharynx; eyespots present; divided lobes of tail less than half length of main stem and constricted off from it; body and tail including lobes covered with minute spines, evenly dis-

tributed; excretory system with V-shaped bladder, ten flame cells in body and two in tail, openings of bladder at tips of divided lobes; between fifty and sixty cephalic glands which distend posterior part of body; body 0.16 mm., stem of tail 0.59 mm., lobes of tail 0.11 mm. in length; in digestive gland of *Planorbis trivolvis* Say from Douglas Lake, Michigan.

The excretory system of *Cercaria elephantis* corresponds exactly to that of *Cercaria douthitti*, having the same number of flame cells in relatively the same position, the same type of bladder with its openings at the tips of the divided lobes of the tail and even the little island (Fig. 1 i) in the same position at the base of the tail. The portion of the bladder in the main stem of the tail is of course longer than in the former species on account of the greater length of the tail. In fact, with this one variation the drawing of this system in *Cercaria douthitti* would do equally well for *Cercaria elephantis*.

Cercaria emarginatae nov. spec.

Forked-tailed cercaria with pharynx and without eye-spots; lobes of tail almost as long as stem and not constricted off from it; number of cephalic glands six, extending into postacetabular region; heaviest spination over anterior tip and around acetabulum, with rest of body only sparsely covered; intestinal ceca extend almost to posterior end of body; excretory bladder V-shaped, ten flame cells in body and four about in mid region of stem of tail; openings of bladder at sides of divided lobes of tail; body 0.16 mm., stem of tail 0.23 mm., lobes of tail 0.20 mm. in length; in digestive gland of *Lymnaea emarginata angulata* Sowerby from Douglas Lake, Michigan.

The excretory system of *Cercaria emarginatae* is shown diagrammatically in Figure 2 B. The excretory bladder corresponds to that of *Cercaria douthitti* except that the openings are at the sides of the divided lobes of the tail. The number of flame cells in the body of this cercaria is ten, arranged along the sides as in the other forms. In the tail there are four flame cells, two on a side, located about the middle of the stem and connected with the posterior collecting tube of the body by a long tubule on each side. A form closely related to *Cercaria emarginatae* was worked out during this past summer by one of my students, Mr. John C. Johnson. The relations of the excretory system in this species correspond to those in *Cercaria emarginatae* except for the position of the flame cells in the tail which was the same as in *Cercaria douglasi*, and for the island at the base of the tail.

Cercaria douglasi nov. spec.

Forked-tailed cercaria with pharynx and no eye-spots; lobes of tail more than half length of main stem and not constricted off from it;

heavy spination over anterior tip and around ventral sucker; scattered spines over rest of body; cephalic glands four, not extending into post-acetabular region; excretory bladder with commissure in front of acetabulum connecting branches; flame cells as in *Cercaria emarginatae* except that those of tail are in its anterior portion; intestinal ceca extend two-thirds of distance between acetabulum and posterior end of body; body 0.14 mm., stem of tail 0.18 mm., divided lobes 0.16 mm. in length; in digestive gland of *Physa ancillaria* Say from the Douglas Lake region.

The excretory system of *Cercaria douglasi* is shown in Figure 2 C. The bladder is quite different from that of *Cercaria emarginatae*, since the sides of what constitutes the V in that species are in *Cercaria douglasi* united by a commissure which makes a triangle with the base in front of the acetabulum. The anterior and posterior collecting tubes on each side enter the bladder at the angles of the base of the triangle. The number of flame cells and their general arrangement is the same as in *Cercaria emarginatae* except for the position of the flame cells of the tail.

GENERAL DISCUSSION

An analysis of the forked-tailed cercariae described above shows that they fall into three distinct groups. The first group might be characterized by the absence of a pharynx, by the fact that the lobes of the tail are less than half the length of the main stem and definitely constricted off from it and by the presence of eye-spots. This group includes of my material *Cercaria douthitti* and *Cercaria elephantis*. The second group, the cercariae of the human schistosomes, is represented by the cercaria of *Schistosomum japonicum*. This group agrees with the first in characters one and two, but has no eye-spots. Group three might be characterized by the presence of a pharynx and the fact that the lobes of the tail are almost as long as the stem and not constricted off from it. The first two groups belong to the family Schistosomidae, but the third group differs from this family in the presence of a pharynx.

A comparison of the excretory systems of these five cercariae shows a remarkable uniformity. The exact correspondence between this system in *Cercaria douthitti* and *Cercaria elephantis* would seem to indicate close relationship and if the adults were known I should expect to find them belonging to the same genus or closely related genera. Here is a case where cell constancy indicated by a correspondence in number of flame cells is carried beyond the species limit. One would hardly expect to find specific differences in the excretory systems of trematodes belonging to the same genus except those produced by differences in size relations. The constancy of the excretory systems

of these two cercariae is all the more striking when their superficial differences are taken into consideration. *Cercaria elephantis* has a tail almost three times as large as that of *Cercaria douthitti* and a very much larger number of cephalic glands. The eye-spots also are much larger in the former species.

A comparison of the type of excretory system found in the cercaria of *Schistosoma japonicum* with the conditions in the first group (Figs. 1 and 2 A) shows that they are homologous except for the number of

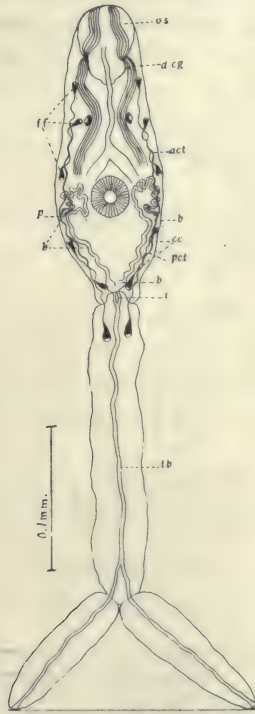


Fig. 1.—*Cercaria douthitti*, ventral view, to show excretory system. Cephalic glands not shown. *act*, anterior collecting tube; *b*, bladder in body region; *cc*, capillaries; *d cg*, ducts of the cephalic glands; *i*, island in excretory bladder; *os*, oral sucker; *p*, point where collecting tubes join bladder; *pct*, posterior collecting tube; *tb*, extension of bladder into main stem of tail.

flame cells. The cercaria of *Schistosoma japonicum* has the smallest number of flame cells that I have ever observed in a fully developed cercaria or seen recorded in the literature.

More striking still is a comparison between these first two groups and the conditions found in the excretory system of the third group (cf. Fig. 2 B and C). The forms belonging to this group have a distinct pharynx, which sets them off very clearly from the first two groups and places them outside the family Schistosomidae. Yet com-

parisons of the excretory systems show striking homologies which must certainly indicate a fairly close relationship between the family to which these forms belong and the schistosomes. Therefore, the knowledge of the life-history of a member of this group such as *Cercaria emarginatae* would help us to understand the relations of the family Schistosomidae to the other digenetic trematodes.

Cercaria vivax Sonsino belongs to the third group. Looss (1896: 210) describes this species fully, but does not show the excretory system sufficiently for the determination of its homologies. He shows six flame cells in the tail, but does not make clear their connections. His

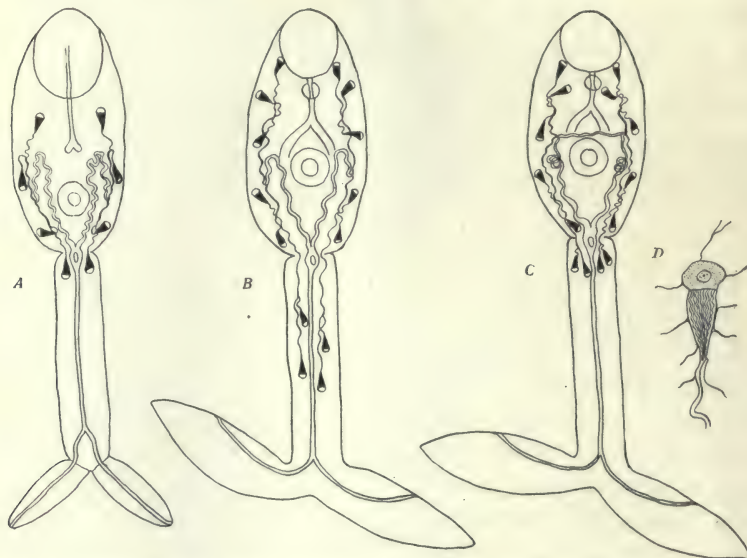


Fig. 2.—Diagrams of the excretory systems of three different forked-tailed cercariae. A, *Cercaria* of *Schistosoma japonicum*. B, *Cercaria emarginatae*. C, *Cercaria douglasi*. D, Typical flame cell of the forked-tailed cercariae. Very highly magnified.

work on the development of this form (Looss, 1896, Pl. xv, Figs. 172, 173) indicates very clearly the double origin of the type of excretory system found in the forked-tailed cercariae. This is suggested in the mature cercariae described above by the complete separation of the flame cells of each side, the division of the bladder in the body, the island at the base of the tail and the division of the bladder to open on the lobes of the tail.

The fundamental homology of the excretory systems of these forked-tailed cercariae indicate that at least in this group the pattern of the excretory system is very conservative. I have in addition a considerable number of unpublished observations on the excretory

systems of the cercariae of other groups of trematodes which supports this view, and tend to extend the principle of conservativeness to other trematode groups. This indicates that studies on the excretory systems of cercariae may go far in establishing the true relationships between the various subdivisions of the digenetic trematodes.

SUMMARY

The excretory system was carefully studied in five forked-tailed cercariae, *C. douthitti* Cort, the cercaria of *Schistosoma japonicum*, *C. elephantis* n. sp., *C. emarginatae* n. sp., and *C. douglasi*. Altho these five fall into three separate groups which represent at least two distinct families, this system shows remarkable uniformity.

In *C. douthitti* and *C. elephantis* the system corresponds both in the arrangement of tubules and in the number of flame-cells, suggesting close relationship for these species.

The excretory system of the cercaria of *Schistosoma japonicum* is the simplest known in a fully developed cercaria and has the fewest flame-cells.

Cercaria douglasi and *C. emarginatae* differ considerably from the other three forms studied. In the presence of pharynges, also, they depart from conditions in the family Schistosomatidae. The similarity of the excretory system, however, demonstrates their relationship to that family.

These observations indicate the conservatism of the excretory system in trematodes and its value in establishing relationships in this group.

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THE MORPHOLOGY AND LIFE HISTORY OF A NEW
TREMATODE PARASITE, *LISSORCHIS FAIRPORTI*
NOV. GEN., ET NOV. SPEC. FROM THE
BUFFALO FISH, *ICTIOBUS**

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During the summer of 1917, while working as an investigator for the United States Bureau of Fisheries at the Fairport Biological Station, my attention was directed to certain ponds for experimental fish culture where young buffalo fry had suddenly begun to die in large numbers. The problem was assigned to me for investigation, and I thank the Bureau for its assistance and for the opportunity afforded.

These fry hatched from artificially fertilized eggs on about May 20, and were divided into three batches. One lot of 36,000 was placed in pond 16 *B*, another of 6,500 in pond 3 *F*, and the third of 2,500 in pond 2 *F*. It was in these latter two ponds that the death rate was so high while in 16 *B* no unusual death rate was noted. Unfortunately, the records on pond 2 *F* are incomplete, but not so many died there as in pond 3 *F*. The death record for 3 *F* is as follows:

July 2.....	331 were found dead
July 3.....	329 were found dead
July 6.....	288 were found dead
Total.....	948 dead in four days' time
A few others died afterwards.	

This is not the first time that large numbers of buffalo fish have been lost in the experimental ponds at Fairport. On May 24, 1916, pond 7 *D* was drawn off and there were found only 1,113 fingerlings of the 19,433 fry which had been put into this pond the previous summer. Only 145 fish had been removed for experimental purposes, so that 18,175 were unaccounted for. It should be noted here that buffalo fish in this pond were infected with the same trematode as found in the dead fry from ponds 2 and 3 *F*, and which will be discussed in this paper.

There was nothing unusual about the conditions of these ponds, and all the ponds got their water supply from the same source. There seemed to be plenty of food and the fish were making good growth. There was no blanket of algae in pond 2 *F*, and this might have

* Contribution from the Laboratory of the United States Bureau of Fisheries, Fairport, Iowa.

accounted in some measure for the fact that there were fewer chironomid larvae in this pond than in 3 *F* where there was a small blanket of mixed algae.

The stomachs of eight fish averaging 4.6 cm. in length were examined and it was found that only two had eaten chironomids, and in these it formed only 2% of the food. Of the six fish averaging 5 cm. in length taken from pond 3 *F* four had eaten chironomids, and in these the larvae made up from 1% to 85% of the total food. Other plankton forms were found in the stomachs of all the fish.

The fish from both ponds presented no unusual appearance when found dead, and no unusual growth of fungus was noted. They were not badly bruised and their gills were comparatively free from silt; no ectotrematodes and but few myxosporidian parasites were found. Upon systematic examination of the fish for animal parasites there was found only one species and this proved to be a new trematode. It was always present in the dead fish and ranged in numbers from one to six or seven per host. These trematodes were immature, very active, and in proportion to the small size of the intestine of the fry occupied a considerable portion of its lumen. Their intestinal ceca were always gorged with food, and the evidence seems strongly in favor of the conclusion that these parasites were in some way responsible for the death of the fry, all the more so as the fry in other ponds were uninfected and did not die in noticeably large numbers. About 50 per cent. of the fry which were living in pond 3 *F* were infected, while only 20 per cent. in pond 2 *F* were infected. These figures were obtained after the fish had stopped dying.

It seems well here to emphasize a point which is often misunderstood. Many times parasites are of themselves not injurious to their hosts, yet their ultimate effect is detrimental. Often typical cases of icterus are produced by the mechanical stoppage of the gall duct by worms which otherwise would not seriously inconvenience the victim. It is not unlikely that in this case the buffalo fish were weakened to such an extent by the parasites that they died from some cause which would otherwise not have affected them. Along these lines of research there is much to be done in the field of parasitology and its importance demands the attention of investigators.

An examination of one and two year old buffalo fish from the experimental ponds showed that they harbored the same parasite in their intestines, and that the percentage of infection was about fifty. Here the adult and immature forms were found upon which, together with material from the fry, the following description is based. It is interesting in the light of what is to follow that in over two dozen buffalo fish taken from the river in the neighborhood of Fairport, none were found infected with this parasite.

The following table shows the degree of infection of one and two year old buffalo fish from ponds 5 and 7 D.

	<i>Ictiobus cyprinella</i>	<i>Ictiobus bubulus</i>
Number examined	13	8
Number infected	6	4
Number not infected.....	7	4
Percentage of infection.....	45	50
Infection in both species, 47%		

A careful study of this parasite has justified the creation of a new genus of which it is the type species. I propose to call the genus *Lissorchis*, and present it with the following description:

MORPHOLOGY OF LISSORCHIS NOV. GEN.

Lissorchis. Body flattened, elongate, tapering posteriorly and of moderate size. Cuticula covered with small spines; fleshy spines around suckers. Acetabulum powerful and as large or larger than oral sucker which is also well developed. Prepharynx and esophagus much reduced; intestinal crura not reaching posterior end. Excretory system Y-shaped, branching anterior to testes. Genital pore marginal and sinistral, situated below middle of acetabulum. Ovary mesial and lobed, no seminal receptacle; Laurer's canal present. Uterus coiled and extending from beyond genital pore to posterior tip of body, filled with small thin-shelled eggs. Testes ovoid, large, mesial and unlobed, lying in line with ovary and posterior to it. Very large seminal vesicle and well developed cirrus anterior to ectal end of uterus. Vitellaria extending on either side from posterior side of acetabulum to half way between posterior tip and acetabulum. Vitellarial sac present and Mehlis' gland large. Habitat: Intestine of fresh-water fishes. Type species: *Lissorchis fairporti*.

It becomes evident that this genus can not be embraced by any of the previously described subfamilies, and so it is here placed as the type genus of the new subfamily Lissorchiinae, the characters of which, must at present, be taken from the genus. If Lühe (1909) is followed this subfamily would be placed under the family Distomata, but according to more recent work these subfamilies have, for the most part, been elevated to the rank of families. If this tendency is followed, it seems necessary to create a new family or to modify the family Plagiorchiidae so as to contain it, but an ultimate decision of this point will not be attempted in the present paper.

LISSORCHIS FAIRPORTI NOV. SPEC.

Altho this worm is very active its activity is confined to the anterior portion of the body so that the part posterior to the acetabulum is practically motionless.

Well extended worms are in the form of an elongated oval (Fig. 1) with the posterior end pointed and the region of the acetabulum widest. The adult mature worms are from 2 mm. to 3 mm. long, altho in life they are capable of extending themselves to 5 mm. in some cases. The average thickness of the worms in the region of the acetabulum is 0.61 mm. The ratio of the anterior to the posterior portion of the body is 1:1.7, altho this is subject to some variation depending upon the degree of extension of the anterior portion.

The cuticula (Fig. 20) of these worms is about 7μ thick and is covered in all regions of the body with spines which do not protrude their entire length thru the cuticula. These spines are most prominent at the posterior end. They are 20μ long, sharply pointed, and have a proximal thickening. Muscular activity of the body serves to extrude them partly.

The oral sucker (Figs. 1, 12, and 13) is large and slightly oval, being 0.38 mm. by 0.41 mm. It is surrounded on its outer margin by small fleshy spines, which occur in two or three rows. In ratio to the length of the pharynx this sucker is 7:5, and in ratio to the ventral sucker it is 7:9. The ventral sucker (Figs. 1, 12, 15) is powerful and round, being about 0.55 mm. in diameter. It, too, is surrounded by several rows of fleshy spines. The muscular pharynx (Fig. 14) leads immediately into the intestinal crura which are generally gorged with food and extend to near the posterior tip of the body, but never entirely so, the uterus lying in the intervening space.

The excretory system (Figs. 2, 19) was studied in living specimens. The pore is situated in the middle of the tip, and from it there leads forwards a rather large median duct, which does not give off branches but is capable of distention. This median trunk divides on the dorsal side of the worm and just forward of the anterior testis. At the base of the two divisions there are slight enlargements, which assume the appearance of a bicornuate bladder during certain stages of the excretory process. Each main lateral branch shortly gives off a branch which passes posteriad and obliquely to near the lateral margin of the worm and then branches into two main stems, one passing up anteriad and the other posteriad; these in turn break up into numerous branches with flame cells at their terminals. The rest of the two lateral branches pass anteriad on their respective sides, giving off branches en route, finally making a loop near the base of the oral sucker, they pass posteriad lateral to the ascending trunks and break up in numerous branches. This arrangement is characteristic and practically no variation of any note was recorded.

The ovary (Fig. 17) lies in a median position a little distance back of the posterior margin of the acetabulum; it is rather deeply lobed, two to four times on each margin. Its length is about 0.36 mm., and its greatest width and thickness about 0.23 mm.

A short oviduct leads from the ovary into the oötype (Fig. 10), and this former comes from the anterior middle of the ovary. From the oötype there arises Laurer's canal, which is a short duct leading to a dorsal pore at a level near the anterior margin of the ovary. Around the oötype is found Mehlis' gland, which is large and lies chiefly towards the ventral side. The oötype continues as the uterus, which characteristically passes forward to a level of the posterior margin of the acetabulum, then takes a sudden turn and runs posteriad along the right side to the posterior tip of the body. Here it forms a complicated coil and then passes anteriad up to the left side to join the prostate near the common genital pore. The uterus is filled with thin-shelled eggs, more or less pointed at one pole where there is a small lid. The eggs (Fig. 9) average 10 by 20 μ .

The vitellaria, or shell glands, are follicular, yellowish-brown structures lying along each side of the body from the posterior margin of the acetabulum to about midway of the posterior region of the body. Minute ducts gather up the secretion and the ducts from each side form a common duct at about the level of the ovary. The common ducts from each side pass into a little sac (Figs. 10, 17), about 8 μ in diameter, which lies just ventral to the ovary and has a minute duct leading into the posterior portion of the oötype.

The testes lie in the posterior portion of the body (Figs. 11, 18), both posterior to the ovary, altho in the oldest specimens the ovary may overlap the anterior portion of the testis. Both testes are ovoid, the posterior testis averaging 0.43 by 0.18 mm., and the anterior 0.49 by 0.17 mm. From the anterior margin of each and towards the dorsum, there pass forward the vasa efferentia. In the region of the ovary they pass in between its lobes. The testes and the seminal vesicle lie in the median line and into the latter the ducts from the testes pass. In older specimens the vesicle (Figs. 11, 16) is filled with spermatozoa, and these are divided into two regions, undoubtedly showing that the germinal products are all expelled at once from a given testis and that the two testes expel their products at different times. The vesicle is ovoid in shape and averages 0.39 by 0.17 mm. It is covered by a thin wall and from its anterior sinistral margin there passes the cirrus sac, crescentic in shape, containing unicellular prostatic glands and, anteriorly, the cirrus, which is extremely thick (Fig. 21). This duct joins the uterus immediately before the genital pore and anterior to it.

LIFE HISTORY

As soon as the infection of the buffalo fry was noted an examination of snails was undertaken, and many of the following species from several ponds were inspected. These represent all the different species found in any great numbers around the ponds: *Lymnaea obtusa*

exigua Lea, *Succinea retusa* Lea, *Planorbis trivolvis* Say, and *Physa heterostrophia*. Of these four species none were found to be infected save certain individuals of *Planorbis trivolvis*, and these from ponds in which there were adult infected buffalo fish. The following table shows the number examined from pond 7 D where the infection was about 10%.

	Number Examined	Number Infected
July 23.....	5	1
24.....	5	0
25.....	4	1
27.....	3	1
31.....	67	5
Aug. 3.....	36	4
Total	120	12

These snails had cercariae (Fig. 3) infecting their livers, and in the water of an aquarium in which these infected snails were kept these cercariae were found swimming about in great numbers.

No counts were made, but it was evident that the entire livers of the snails were filled with this cercaria, and that there were perhaps several thousands in each host. The cercariae were very active and moved about very rapidly. There seemed to be a tendency for them to throw off their tails, and they were often seen in that condition when they became sluggish.

The tail (Fig. 25) of the cercaria is not quite so long as the body, and in the illustration (Fig. 3), which was made from life with the cercaria moderately extended, the tail was 0.27 mm. long. It is not perfectly smooth, but here and there shows indentations. The body of the worm is oval elongate and rounded anteriorly; the length and width vary with the degree of contraction, but in the illustration is 0.40 mm. long and 0.14 mm. wide in the acetabular region. The whole body is covered with spines and the two very prominent suckers are surrounded with them.

The oral sucker (Fig. 22) is about 72μ wide and has a very small stylet (Fig. 8) set into its anterior margin. This interesting organ is in the shape of a pen point and 18μ long and 4μ wide at the base. About one third of the length from the anterior end there is a thickened expansion. The ventral sucker lies two thirds of the distance from the anterior tip of the body and is about the same size as the oral, but more nearly circular in shape as seen from the ventral or dorsal surface.

The stylet glands (Figs. 3, 23) lie anterior to the acetabulum and are in right and left sets. In each set are from four to six fairly large cells that have ducts leading anteriorly to open near the base of the stylet. Cystogenous gland cells are numerous over the body and are found chiefly posteriorly.

The digestive tract is already well developed and has a small pharynx, esophagus and diverticula. These latter do not reach to the end of the body and in most cases do not extend below the acetabulum.

The excretory system is well developed (Fig. 3). There is a bicornuate bladder some distance from the anterior end of the tail, from which two divisions pass anteriorly. A short distance from the bladder they each give off a large branch which passes posteriorly, and these in turn divide into anterior and posterior ducts. The remainder of the main divisions pass anteriorly giving off branches until near the level of the oral sucker they turn back on themselves and pass posteriorly, ending near the middle of the body. A branch passes from the bladder posteriorly and has the excretory pore on it at the posterior end of the body. Then a branch passes out into the tail from this pore as a median caudal duct. Excretory granules were not noted in the tubules.

Nothing of the nervous and reproductive systems could be made out in the living material, but in preserved material one could see a small group of cells just posterior to the acetabulum, the first trace of the ovary (Fig. 24). No indication of the male genital system was noted.

In certain snails, apparently those which had not been very long infected, there were found non-motile sacs (Fig. 7) which contained the developing cercariae, and in some instances the cercariae themselves. One sac, 1.3 mm. long and 0.23 mm. wide and bluntly rounded at both ends, was found from which all the cercariae had escaped save one. Another sporocyst which contained no fully developed cercariae was 0.71 mm. long and 0.12 mm. wide. In this the germ balls were ovoid in shape and the largest ones were 0.10 by 0.05 mm.; there were about fifty such germ balls.

Three attempts were made to infect buffalo fish directly with the cercariae. The method was to make a suspension of cercariae in water and to inject the fish with it. In the case of the experiments with fry the cercariae were placed in an aquarium with a little water and the fry allowed to remain in this for several hours; controls showed that they would eat the cercariae or at least take them in with water. The first attempt was made on July 23, when four buffalo fish from the river were injected. The next attempt was made on July 27, when three one-year-olds from pond 7D and three adults from the river were injected, and the final trial was made on July 31, when twelve fry from pond 4D were fed cercariae. Controls were kept and the fish were killed at varying periods of time. In all the experiments no indication of experimental infection was noted. None of the river fish were infected, and the few infected pond fish had infections of such long standing that it was impossible to consider them as being experimental.

Indeed, controls killed a few hours after being injected showed clearly that the cercariae were being digested, and that this digestion was going on in the stomach. The evident conclusion was that the normal infection is not direct and a secondary intermediate host was sought.

In previous studies it has been shown that the forms of plankton which figure most in the food of the buffalo fish are Chydorus, Daphnia, Cyclops, Diaptomus, Diffugia, Bosmina, rotifers and chironomid larvae. The cercariae were larger than any one of these forms, with the exception of the largest rotifers and chironomids, but for experimental control reasons they were placed in a watch glass with these plankton forms and allowed to remain together for several hours. At the end of the time examinations were made of many individuals of the different species and of these, the cercariae had not attacked any save the chironomid larvae. Every one of the larvae in the dish had just beneath the skin from one to seven cercariae encysted (Fig. 4) in spherical cysts. A few days later this experiment was repeated, and this time the cercariae were seen to attack the larvae actively, and by the use of their suckers and powerful movements of the tail they bored their way into the larvae. The stylet was evidently used in the process and the lashing of the tail served to spin the cercaria in much the same manner that an auger is used. The time required for the process averaged about twenty minutes. Several species of chironomids were tested and no preference was noted. The only species identified were *Chironomus lobiferus* Say and *Tanytus decoloratus* Malloch, but other species of larvae were observed to become infected. The cercariae dropped their tails before they had completely entered their host, and when they had entered discharged their cystogenous substance, rolled themselves into a ball, and remained just beneath the skin of the larvae, showing very little motion in the cyst. The stylet remained in place. After several hours excretory granules were seen to accumulate in the posterior part of the cercaria, and after twenty-four hours quite a portion of the posterior excretory tube was filled.

Two series of experiments were undertaken to infect buffalo fish with the infected chironomid larvae. The first of these was begun on July 31. The livers of three infected snails were teased out in a little normal saline and added to an aquarium containing many thousands of chironomid larvae. These were allowed to remain from 11 a. m. to 3:30 p. m., at which time they were examined, and it was noted that nearly all of the larvae had encysted cercariae in them. Twelve buffalo fry and three yearlings were then put into the aquarium. At 5 p. m. one fry was examined; it had not eaten chironomids and was uninfected. Twenty hours after feeding, another fry was examined, and this one had eaten several larvae, which were partly digested. In the intestine of this fish were four cysts containing cercariae, which were

very active and contained a large mass of excretory granules (Fig. 5). Under the microscope one of the cercariae burst its cyst, expelled its granules and its stylet and assumed the shape and appearance of the definitive form. One partly digested chironomid still had a cyst in it. On August 1 three fry were examined and one yearling; the yearling had two young trematodes in its intestine, which were like in every respect the young trematodes taken from the fry which were infected in pond 3 F. The three fry had not eaten the larvae and were not infected. The rest of the fish were examined on August 2, only one fry being infected, and this one had still the remains of the chironomids in the lower part of the intestine. One or two of the other fish had eaten the larvae, but perhaps had not gotten hold of the infected ones.

On August 3 the experiment was repeated on six fry from pond 4 D where the fry were not infected, and four yearlings from pond 7 D. This time less water was used in the aquarium in which the fish were fed, and they were allowed to remain longer with the infected chironomids. Three fry and two yearlings were used as controls, and these were uninfected at the end of the experiment. Four out of the six fry were experimentally infected and three out of the four yearlings. In this lot various stages were found, from the still intact cysts to worms as large and larger than those found in natural conditions. One yearling examined on August 11 had three trematodes which gave the following measurements: (a) 0.80 by 0.30 mm., (b) 0.90 by 0.30 mm., (c) 1.41 by 0.41 mm.

Figure 6 shows one of these worms drawn from life, and in this the excretory system is identical with that of adult worms and is but a further development of the condition in the cercaria. The ratio of the size of the suckers, shape of the body, spinous skin and general movements of the body are alike in the young forms, adults, and cercariae. The immature forms obtained from the experimentally infected fish are exactly like those found in natural infections, their organ systems being carefully compared. In the development of the reproductive organs the ovary appears first, the two testes next, and the seminal vesicle last, altho the latter seems to be well developed as soon as the testes are ripe.

Attempts have been made to hatch the eggs from adult worms and thus to infect the snails with the miracidium, but so far none of the eggs have hatched.

In the light of the above experiments and observations an explanation might be offered as to why the river fish are not infected. *Planorbis trivolvis* is a very common snail around the ponds at Fairport, but does not seem to be found in the river near this station. The author has looked for it to some extent and so have others with negative results. This species of snail is common in some rivers, and no

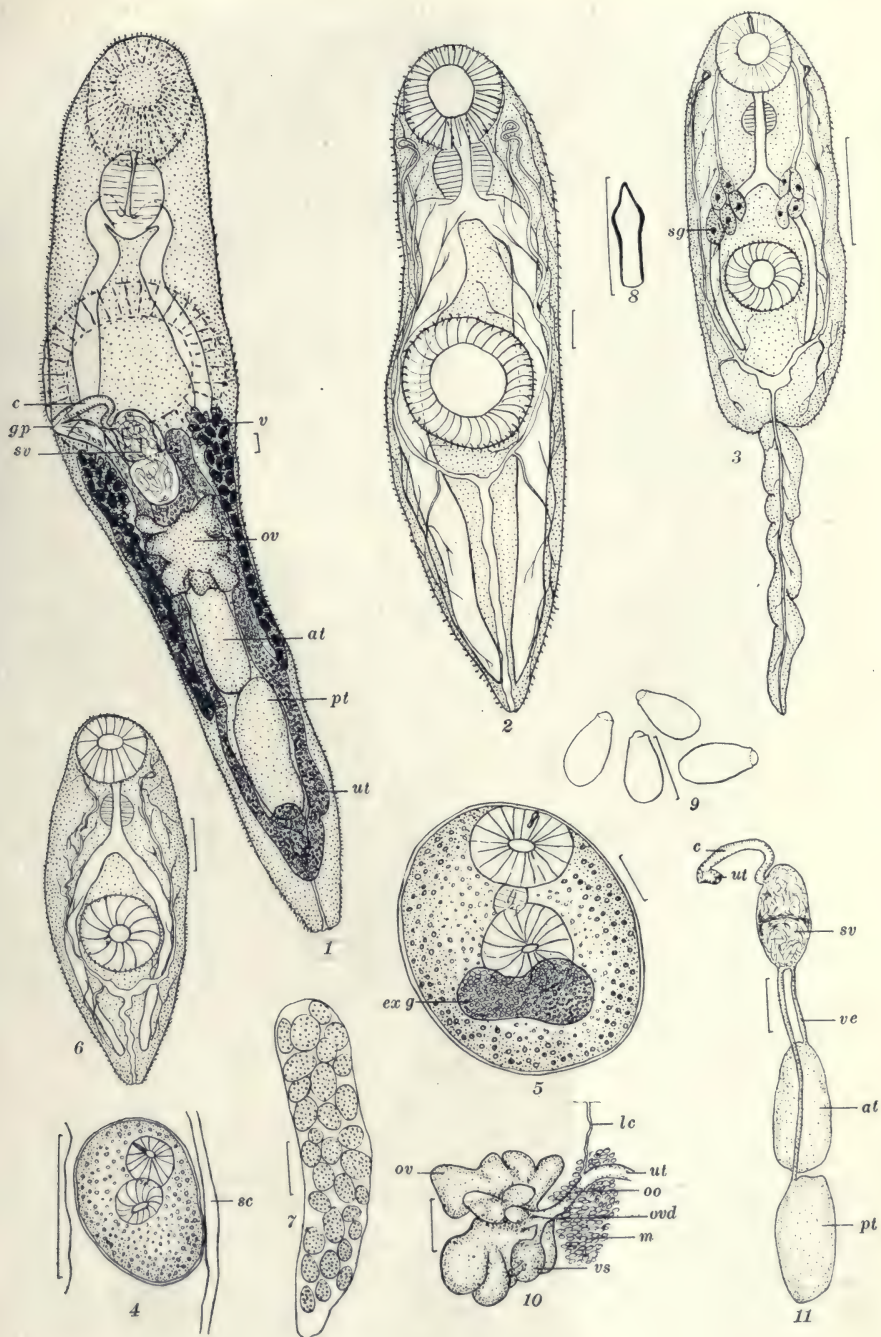
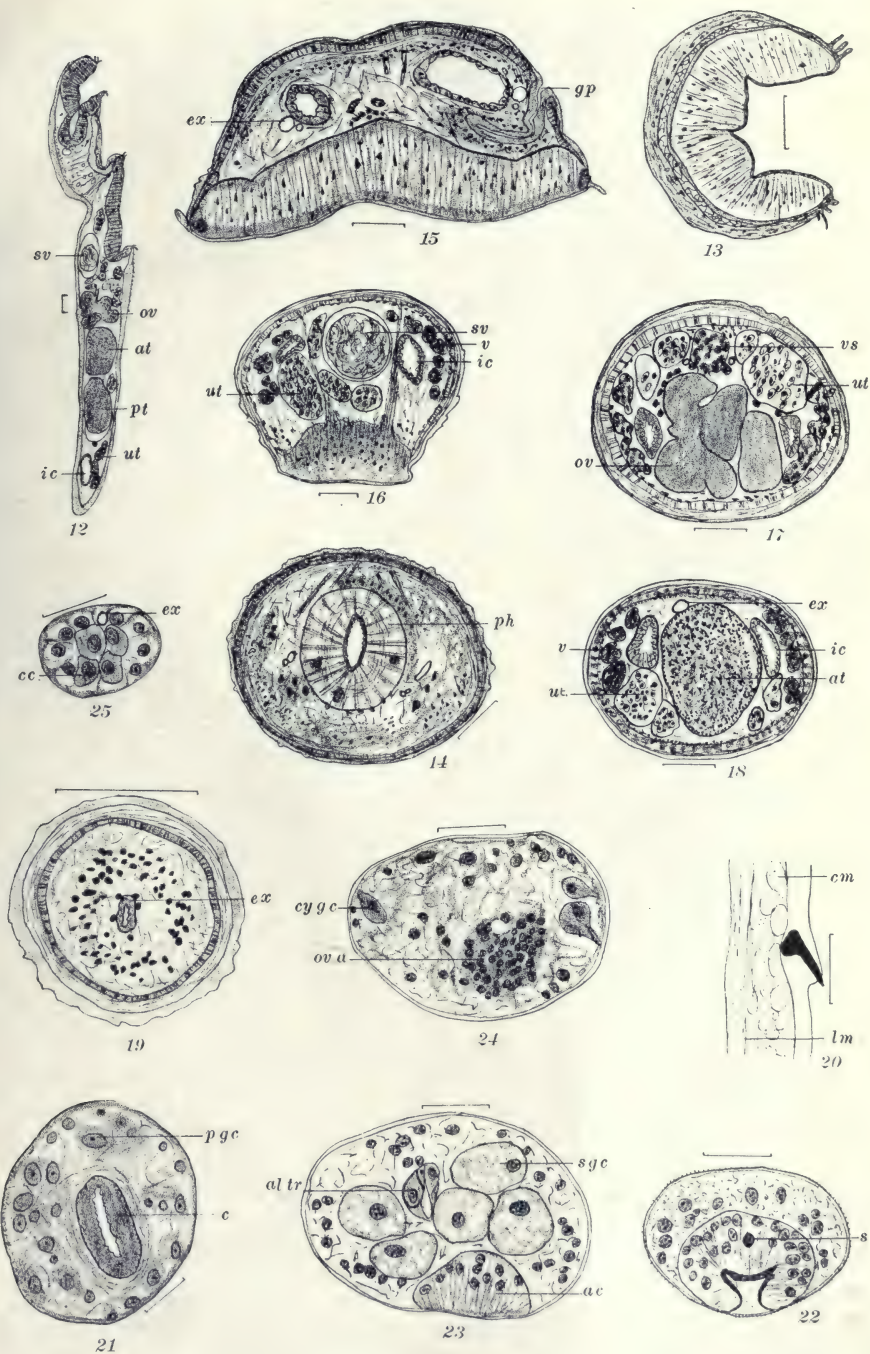
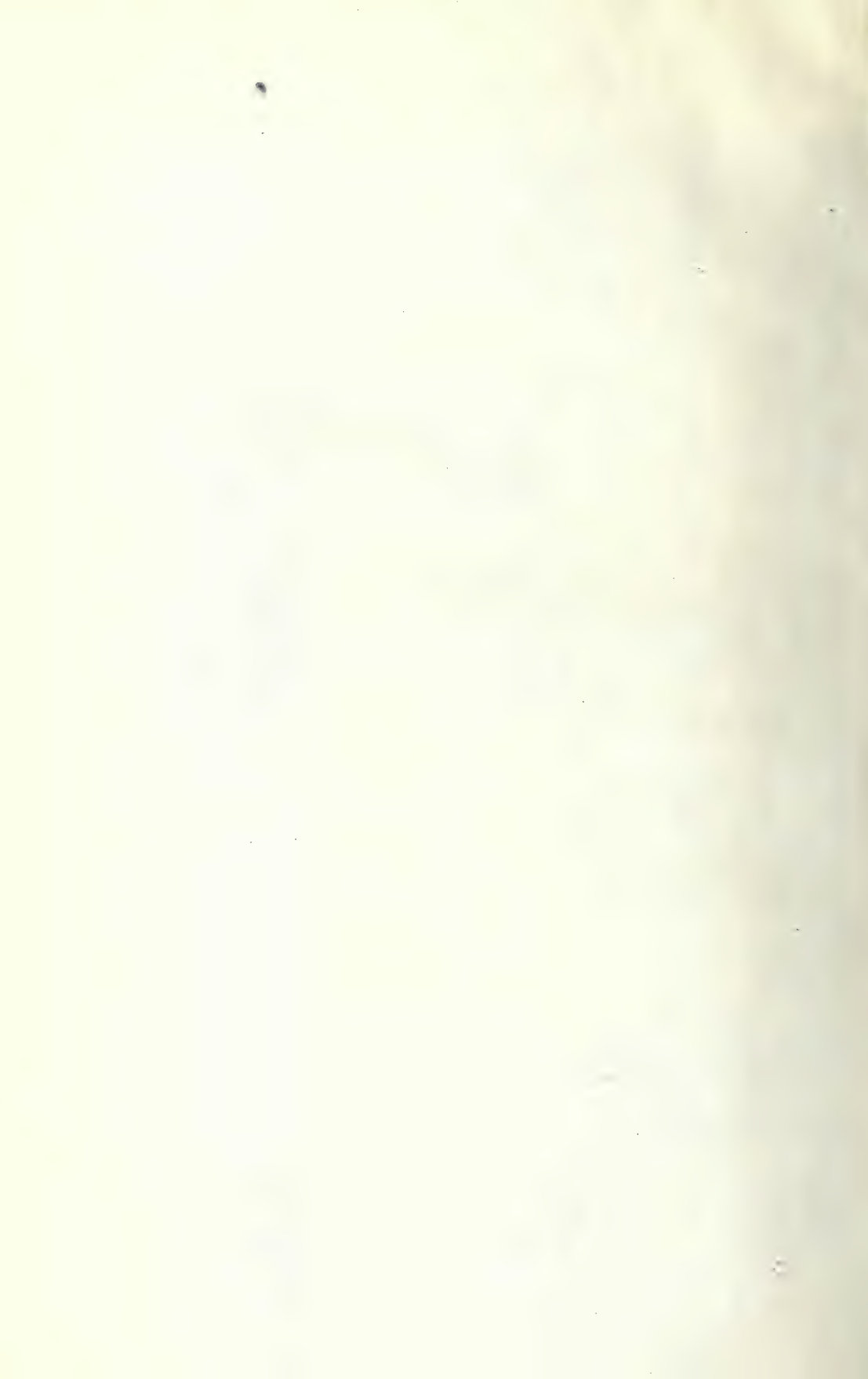


PLATE I







explanation can be offered as to why it is not found around Fairport. While some species of cercaria are known to occur in several snails, this is not always the case, and there is no evidence so far that the cercaria of *Lissorchis fairporti* occurs in any other snail.

It seems desirable to keep young buffalo fish free from this infection and the means of doing it is not so difficult as it might appear. The problem is obviously involved in keeping the fish pond free from this species of snail, and fortunately *P. trivolvis* carries its eggs on its back, so that if a screen which would keep out the snails themselves were provided around the pond and over the inlet pipe of the water supply, there would be no danger of the eggs being brought in, and as a rule there does not seem to be a very marked migration of these snails. Of course, the free cercariae or encysted cercariae might be brought in with the water, but the cercariae do not live very long outside of a host, and this fact would favor prevention of infection. It appears from the evidence at hand that after the fish have grown past three or four inches long they are fairly safe, and hence they would not have to be protected for more than a year. The author has started some experiments along this line and also on the effect of the parasite on the buffalo fry which he hopes to bring to a definite end in the near future.

In the examination of infected fish it was noted as the summer advanced that the average size of the trematodes in the fish was greater than at the beginning of the summer, that more eggs accumulated in the uteri of the worms, and that toward the last of the summer they began to expel them in great numbers. In the early summer there were more sporocysts found in the livers of snails than in the later summer, which is correlated with the previous statement as might be expected. From these observations and the experimental evidence, it seems likely that the miracidium hatches out from eggs laid in the late summer and finds its host, the snail, before the winter sets in; that it develops in the liver of the snail during the winter and spring, and in the first part of the summer the cercaria leave their hosts. The infection of the chironomids and fish naturally takes place during the later part of the summer. It is barely possible that some of the first to infect the fish mature before fall, but it is more likely that they all live over winter in the fish before they become mature. The facts that the eggs are in the last stages of cleavage in the late summer and the infection of the snails is so far advanced by early summer, support the conclusion that the miracidium is hatched in the fall and lives over winter in the snail.

The lower percentage of infection in pond 2 F is explained by the fewer chironomids in the pond and the smaller percentage found in the food bulk of the fry in this pond than in the case of the fish in pond 3 F.

SUMMARY

1. An adult trematode, found in the intestine of *Ictiobus cyprinella* and *Ictiobus bubulus* from experimental ponds at the United States Bureau of Fisheries Biological Station at Fairport, Iowa, is shown to be a new species, *Lissorthis fairporti*, type of a new genus and new subfamily. About 50 per cent. of these fish are infected.

2. This parasite appears to be responsible for the large death rate in young buffalo fish in these ponds. It is not found in fish of the same species taken from the Mississippi River near Fairport.

3. The cercaria of this form has been found in *Planorbis trivolvis* and described; it belongs to the xiphidiocercariae. It encysts in chironomid larvae after boring thru their skin, and when these were fed to buffalo fish the worm was freed from the cyst and developed to stages like those found in nature in infected fish. Eggs from the adult trematodes probably hatch in the late fall and live over winter in the liver of *Planorbis trivolvis*. In the summer the cercariae find their way to water, infect chironomid larvae and in turn the fish. The absence of this snail from the river in the region of Fairport undoubtedly explains the lack of infection in the buffalo from that source.

4. Buffalo fish cannot be infected directly by feeding or injecting the cercariae into their stomachs.

5. Protecting the ponds in which young buffalo fish fry are being raised from *Planorbis trivolvis* would without doubt lower the infection among the fish.

The author wishes to express his thanks to Prof. Henry B. Ward for suggestions in regard to this manuscript.

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ABBREVIATIONS USED IN THE PLATES

<i>altr</i> esophagus	<i>ova</i> embryonic ovary
<i>at</i> anterior testis	<i>ovd</i> oviduct
<i>c</i> cirrus	<i>pgc</i> prostate gland cells
<i>cc</i> central cells of cercaria tail	<i>ph</i> pharynx
<i>cm</i> circular muscles	<i>pt</i> posterior testis
<i>cygc</i> cystogenous gland cells	<i>s</i> stylet
<i>ex</i> excretory canal	<i>sc</i> skin of chironomid larva
<i>exg</i> excretory granules	<i>sg</i> stylet gland
<i>gp</i> genital pore	<i>sgc</i> stylet gland cell
<i>ic</i> intestinal crura	<i>sv</i> seminal vesicle
<i>lc</i> Laurer's canal	<i>ut</i> uterus
<i>lm</i> longitudinal muscles	<i>v</i> vitellaria
<i>m</i> Mehlis' gland	<i>ve</i> vas efferentia
<i>öö</i> oötype	<i>vs</i> vitellarial sac
<i>ov</i> ovary	

PLATE I

Fig. 1.—Dorsal view of *Lissorchis fairporti*, showing genital systems. The spinous condition of the integument should be noted.

Fig. 2.—Ventral view drawn from life, showing the details of the excretory system.

Fig. 3.—Cercaria drawn from life as seen from the ventral side, showing alimentary canal, stylet glands and excretory systems.

Fig. 4.—Encysted cercaria in a chironomid larva. Drawn from life.

Fig. 5.—Encysted cercaria, freed from a chironomid by the action of digestive fluids and found in a buffalo fry's intestine twenty hours after feeding infected chironomids. Drawn from life.

Fig. 6.—Young trematode from a buffalo fish experimentally infected three days before examination. Note excretory system and further development of the digestive system.

Fig. 7.—Sporocyst containing developing germ balls. From the liver of *Planorbis trivolvis*.

Fig. 8.—Stylet of cercaria.

Fig. 9.—Eggs of *Lissorchis fairporti*.

Fig. 10.—Reconstruction of the main parts of the female genital system drawn from a lateral view.

Fig. 11.—Reconstruction of the male reproductive system drawn from a dorsal view.

PLATE II

Fig. 12.—Sagittal section thru the midline of an adult, showing the relation of organs.

Fig. 13.—Cross section thru the oral sucker. Note the fleshy spines around the margin of the sucker.

Fig. 14.—Cross section thru the pharynx.

Fig. 15.—Cross section thru the ventral sucker at the level of the genital pore.

Fig. 16.—Cross section thru the seminal vesicle.

Fig. 17.—Cross section thru the ovary at the level of the vitellarial sac.

Fig. 18.—Cross section thru the anterior testis.

Fig. 19.—Cross section thru the posterior region of the body, showing the median stem of the excretory canal.

Fig. 20.—Details of the body wall showing spine.

Fig. 21.—Details of the cirrus, showing unicellular prostate glands.

Fig. 22.—Cross section thru the oral sucker of a cercaria.

Fig. 23.—Cross section thru the anterior region of the acetabulum showing the stylet glands.

Fig. 24.—Cross section thru the embryonic ovary of a cercaria.

Fig. 25.—Cross section thru the tail of a cercaria. Note the central cells.

The individual scale drawn by each figure indicates 0.1 mm. in all figures except that it is 0.02 mm. long in Figures 5, 8, 9, and 20 to 25 inclusive.

INSECT TRANSMISSION OF INFECTIOUS ANEMIA OF HORSES *

C. W. HOWARD

Swamp fever, or infectious anemia of horses, has been known in Minnesota for some years. At one time it was epidemic and while not at the present time taking that form, is probably more widely prevalent than suspected, because of the ability of the disease to assume a mild, unrecognized form.

The disease is widely distributed, having been recognized in Europe, Japan, recently by Theiler in South Africa, Panama Canal Zone, Canada, and in the United States from Wisconsin, Minnesota, North Dakota, Wyoming, Nevada, Washington, Colorado, Oklahoma, Nebraska, Missouri, Arkansas, and Mississippi.

The name would indicate that it was a disease of low lying, wet land. While this is, generally speaking, true, it is not always so. The European investigators particularly do not emphasize this fact, but have shown that horses pastured near forests are more subject to attack. The disease has been found at an elevation of 7,500 feet, and in many places where the soil was for the most part light and dry. In Minnesota most of the cases seem to have come either from the Red River Valley where the land is mostly low and level, of the prairie type, and the soil rather heavy, there being many swampy areas, or from the northern, undeveloped part of the state where there is much virgin timber and unreclaimed bogs and marshes. But other foci of the disease have been found in parts of the state where the land is almost entirely under cultivation with very little if any forest, and the soil of a light sandy nature. The swamps in these latter places are much restricted, and comparatively few in numbers, but still some are present.

The disease usually appears in early summer, increasing from July until October, and being recognized most widely during August and September. The incubation period seems to vary from ten to forty-five days, the usual time being twelve to fourteen days.

No other animals than equines seem to be susceptible. The Japanese investigators have claimed that the pig is slightly susceptible, and that young goats and sheep show slight febrile reactions, but other workers have not confirmed these results. This inability to employ any of the smaller laboratory animals has considerably hampered investigations, especially on the etiology of swamp fever.

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NATURE OF THE VIRUS

The cause is an ultra-microscopic, filterable virus present in the blood. This may be transmitted to other horses by injections of blood serum which has been passed thru a filter. There are few, if any, symptoms specific for this disease, making it very difficult to recognize with complete positiveness, except by subinoculations of blood. This, together with the fact that only equines are susceptible and the impossibility of knowing when a horse may have had a mild attack of the disease and be immune to further infections has been a severe handicap in prosecuting investigations upon methods of transmission.

The virus seems to easily withstand freezing, but heating to 58° to 60° C. for one hour destroys it, as does bright sunlight. Drying at room temperature in a vacuum does not alter its virulence.

SYMPTOMS OF THE DISEASE

The most characteristic symptom is a progressive anemia without any apparent cause. This is accompanied by a recurrent fever at which time the temperature may rise to 103° F. or higher, dropping nearly or quite to normal after a short run. If worked, the horse tires very easily and may later develop a staggering gait. It may improve for a time only to be followed by a more severe attack. Death may result, or the animal may recover, under which circumstances the blood remains infectious for a long period. In Japan it was found that recovered horses remain virulent for as much as four years. A longer incubation period was required when such blood was used for subinoculations as it gradually lost its virulence with the lapse of time. Van Es claims thirty-five months as the limit of infectivity of recovered horses. A case under observation at the Minnesota Experiment Station still carries virus in virulent form nearly six years after recovery. It may also happen that the infected animal does not show any obvious symptoms of the disease, so mild is the attack. These virus carriers are a source of considerable danger in any region where attempts are made to control the disease.

POSSIBLE MODES OF TRANSMISSION OF VIRUS

The method by which the virus is transmitted from animal to animal is one of prime importance and has been the subject of considerable investigation. Various methods have been suggested. Horses kept in the stable side by side for months may never contract the infection one from the other. In the pasture, however, it is usually able to spread readily, altho some observers claim that it is non-contagious even there. The suggestion that it may be conveyed in water and food seems untenable owing to the destructive effect which

sunlight has upon the virus. It has also been found difficult to give the infection thru the digestive tract, even when very large quantities of virulent blood or urine were fed. The droppings and saliva do not contain the virus, but the urine does. Soiled grass or water would hold only a relatively small quantity of urine. In the stable the virulent secretions would be more cumulative and not subject to the destructive action of the sunlight, yet transmission in the stable is more difficult than in the open. Even the fact that the urine is infectious is, however, disputed by many workers. Vallée and Carré in France, Van Es in North Dakota, the Japanese Commission, and Theiler in South Africa make positive claims. Swingle in Wyoming had negative results to all of his investigations on this question. The positive side, however, agrees that large quantities must be fed to produce the disease.

With these facts in mind it would seem that infection with swamp fever thru the alimentary canal was, at least, not the usual method. There is nothing to indicate infection thru the respiratory passages. The only other method by which the virus could gain entrance to the body would be thru the skin. For virulent blood to enter thru abrasions of the skin would be rare. For this reason blood sucking arthropods have been suspected for some time and some investigations have been carried out, none of which, however, have seemed very conclusive.

REVIEW OF PREVIOUS INVESTIGATIONS

Francis and Marsteller (1908) record that in Texas they kept a healthy susceptible horse all summer and fall in the same pasture with infected horses and never obtained a transmission, altho many varieties of blood sucking flies were present most of the time.

Several observations on the insect transmission of swamp fever of horses were made by the commission appointed by the Japanese government in 1909 to investigate this subject. They noticed that the disease seemed to be easily transmitted in the field but not so in the stable, and drew the conclusion that insects must be the transmitting agent. To test this observation they erected three enclosures each large enough to hold several animals comfortably. One was enclosed with wire screening, the others were simply enclosed with a high board fence. The fenced enclosures were 12.7 meters apart. In the screened enclosure were placed both infected and noninfected horses. In one of the fenced areas were placed nine infected horses and in the other four healthy horses. Some of the noninfected horses in the screened enclosure contracted swamp fever, but only after very long incubation periods, in one case of six months. Those in the fenced enclosures showed the first symptoms of the disease one month after exposure.

They had not come into contact with infected horses, but blood sucking insects had free access. The test was repeated with very similar results, and a third repetition gave still more conclusive results. In each case the experiment ran thruout the entire summer. Their contention was that these results prove that the disease is easily transferred when animals are not in contact but when free access for insects is possible. A shorter incubation period follows than when infection takes place in the stable and the infection is as virulent as in mixed pasture infections. Consequently, they conclude that transmission of the disease is accomplished only thru the agency of insects which are capable of flying.

The Japanese Commission gave very careful consideration to the various blood sucking flies and tried experimnets with some, but in their report do not give any details of how the experiments were carried out. Mosquitoes they claimed were present only in the stables and as stable transmission was rare had to be excluded from consideration. At the time when *Simulium* "monopolized the pasture," no infections occurred. Ticks were excluded because of their inability to move over long distances. *Stomoxys calcitrans* was abundant and there was every chance for it to act as the carrying agent, but they felt sure that it was not. They tried to feed the flies on the horses by hand, but negative results followed. Many flies were kept in the screened enclosure where there were confined healthy and infected horses. One healthy horse developed a doubtful case after an incubation period of six months, so they excluded *Stomoxys* from consideration. Tabanids were thought to be the guilty agent, because of their great numbers, violent attacks upon the horses, and because the seasonal appearance of the disease coincides in Japan with the appearance of the flies. They were, however, unable to handle the flies successfully, so no experiment was tried.

Bots (*Gastrophilus intestinalis*) were also taken into consideration, because of the possibility of the larvae taking up the virus and passing it on thru the adult and egg to the next generation. Several bots were taken from a patient which had died of the disease, crushed, filtered and injected into healthy animals, but results were negative. The next season they freed all their horses from bots before putting them into the pastures, but it had no influence upon the results. K. R. and R. Seyderhelm (1914) took up this line of investigation in Germany.

In Wyoming, Swingle (1912) tested the ability of certain tabanids to act as carriers. He used a large screened cage capable of holding three horses. Large numbers of tabanids (spp. not given) from time to time were let into the cage with a sick horse. He experienced considerable difficulty in keeping them alive in the cage and in getting them to feed, but at one time fifty and at another sixteen and lesser

numbers at other times were observed to bite the horse. He endeavored to persuade these flies to feed on a second susceptible horse, but failed with the exception of one fly. While his experiments gave negative results, Swingle suggested a way in which tabanids might act as transmitters of swamp fever. He noticed that while feeding many flies voided fresh blood onto the skin of the horse, and that this apparently undigested blood was voided as long as three days after feeding. He thought that such blood voided onto grass might be eaten by horses and infection acquired. The difficulty of securing infection thru the digestive tract even with large quantities of infective blood would seem to preclude such a probability. There is a possibility, however, that this voided blood might enter the wound made by the proboscis and mechanical transmission be produced in this way as well as by blood on the mouth parts.

The investigations in Wyoming were continued by Scott (1914). He used a cage large enough to accommodate five horses. Adult mosquitoes, species not given, were collected and placed in the cage. Some "wild flies" (tabanids) were unavoidably brought in from the swamps with the mosquitoes, but died in the cage quickly. House flies and *Stomoxys*, however, survived and bred in the cage. During July the mosquitoes were most abundant. In July a sick horse was alternated with well horses, each being left in the cage for several days at a time. Mosquitoes were repeatedly observed to feed upon the horses, but the results were negative. During August when *Stomoxys* and *Musca domestica* were more abundant in the cage the experiment was repeated and *Stomoxys* was seen to bite the horses several times. One of the three healthy horses used, showed a rise of temperature, but did not react again. A second horse, after three recurrences of the temperature rise, died on October 5, and subinoculation of the blood produced typical cases. The third horse gave no reaction. These results do not, however, seem to us to be conclusive. In the first place there were several varieties of flies in the cage; during the second part of the experiment the healthy horses employed were the same as those which had been exposed to the mosquito bites during the previous month, and then there seemed to have been no provision made to prevent infection by contact during the latter part of the test, as the healthy horses were kept in the cage during the entire course of the experiment, even when the infected horses were present.

A third variation of this test was carried out, by transferring flies from the first cage where they had fed on infected horses, to a second new cage where were one survivor of experiment one and two other healthy horses. The results were negative. Scott thought the reason was that the nights at that time in August and September were very cold, frost occurring several times.

Further experiments have been carried out by Scott in Wyoming with mosquitoes, *Stomoxys*, and tabanids, but the results have not yet been published. He claims that *Stomoxys calcitrans* is the carrier in Wyoming.

EXPERIMENTAL WORK

An extensive study of swamp fever has been made at the Minnesota Experiment station by Dr. C. F. Flocken, who was stationed there for that purpose by the U. S. Bureau of Animal Industry. In the spring of 1915 work was begun by the writer on the insect transmission of the disease, in cooperation with the Bureau.

A careful survey was first made to ascertain exactly what biting insects or insect allies were present where the disease was found and whether or not they fulfilled the following required conditions to make them possible transmitting agents. Such an agent must be found over a very wide area; it must be a ready feeder on the horse and be able to pass rapidly from one to another. The fact also that swamp fever in Minnesota usually begins to appear in July, increasing in August, the incubation period being from ten to thirty days, made it appear that the early spring breeding forms were more likely to be the carriers.

Ticks are represented in Minnesota mainly by *Dermacentor variabilis*, which does not attack the horse extensively and is not found abundantly in all localities, so that it can be eliminated. Species of *Simulium* are present and appear early in the season, but not so early as the tabanids, and are not present in all parts of the state. *Chrysops moerens*, *C. striatus*, and *C. cuclux* are quite common over most of the state but appear only in late July and August.

Mosquitoes are present everywhere. The early spring breeders such as *Aedes fuscus*, *A. auroides*, *A. canadensis* and *A. sylvestris* are especially abundant in our swamp fever areas, but other species are plentiful thruout the summer and autumn. They will be found inside of stables as well as in pastures, but stable infection seems to be rare. If mosquitoes were the agent of transmission swamp fever would probably be more widely epidemic. The same can be said for *Stomoxys calcitrans*, except that it is not abundant in early summer and in that respect does not coincide with the requirements set for possible carriers. It is also found in stables.

Several species of *Tabanus* appear in spring and early summer, during open seasons coming as early as late May, but never later than early July. The early spring forms are *T. lasiophthalmus*, *T. affinis*, *T. zonalis*, *T. illotus* and *T. septem trionalis*.

Other forms appear during mid and late summer, such as *T. longitudinalis* and *T. atratus*, but in very small numbers. These early tabanids are the worst insect pest of horses and cattle in the northern

half of the state, where much of the land is still virgin timber and muskeg, or at least still undrained and swampy. These seemed to fulfill the conditions which were sought, and it was decided to begin work with these horse flies.

A cage 12 by 24 feet was constructed and divided into three equal parts. One end apartment was cut off from the rest by a partition of fine wire netting, insect proof and intended for a check horse, if desired. The other two parts were separated only by a double partition of half inch mesh wire, to keep animals from coming into contact, but to allow of free movement for insects. The roof was covered with screening and the floors were cement, so that the enclosure could be kept perfectly clean. Two screened vestibules, of a size to allow the passage of a horse gave admission to the two parts of the cage. This cage was constructed on one side of a low one story building containing a series of stables, one of which opened into each part of the cage, so that both animals and insects could seek shelter inside if they wished.

It was impossible to breed flies for the work, but it seemed feasible to collect them and place them in the cages. Altho a journey of 100 to 150 miles had to be made for each lot, it was possible to get a large quantity of flies safely to the laboratory. They were usually collected during the late afternoon from cattle or in stables. From 300 to 500 were placed in each traveling cage. Dilute honey and water was provided as food. The collector chose night trains, if possible, in order to avoid the excessive heat of day trains at that time of year. Consequently, half to three-quarters of the flies were active upon arrival at the University Farm. The first lot of flies let loose in the cage perished by beating themselves to death on the netting. After this we resorted to hand feeding. The flies were kept in an outdoor insect breeding cage, 4 by 4 by 6 feet, supplied with a pan of water and grass, and a small tree. Each day these were removed and fed on the horses. Even with this arrangement only three lived long enough to feed more than once, one of these three times, and the other two twice. They would, as a rule, however, feed readily on syrup or dilute honey, but all died in a few days.

In the known cases of disease transmission by tabanids it occurs in a purely mechanical way. This fact was kept in mind. Horse 25 was the source of virus; he had had a severe attack of the disease four years previously and still carried virulent virus; in fact, three months after this experiment, blood from this horse reacted promptly on injection into another horse. Horse 32 was healthy. Flies were allowed to feed on Horse 25 for about one minute, then removed and transferred quickly, with only five to ten seconds interval to Horse 32, where they finished feeding. The process required, altogether, three to five minutes. Hand feeding was difficult, only a comparatively few being

willing to feed under a glass cylinder. Sometimes gentle manipulation of the fly's head with the finger would suffice to give the start. In all, 69 *T. lasiophthalmus* and 2 *T. affinis* fed in this way. Feeding of the flies began on June 26 and ended July 12. Horse 32 was left until October 2, during which period no rise in temperature was noted. On the latter date it was inoculated with active virus and reacted in eleven days, dying on October 29, thus showing that the flies had not carried virus to this horse.

Objections may be raised to this experiment on the grounds that Horse 25 did not have an acute form of swamp fever during the course of the experiment, and that the virus in the blood was not sufficiently active for the small quantities carried by the flies to cause any reaction. However, in nature there must be many such cases responsible for the spread of the disease, and it would seldom happen that as many as seventy-one flies would complete an interrupted feeding on two horses. Then the blood proved to be active upon subinoculation into another horse after the experiment was over.

It was intended to duplicate this experiment during the spring of 1916, using a newer case for the reservoir. A succession of unusual seasons, however, had reduced the number of horse flies so as to make it impractical to secure a sufficient quantity for the purpose. Consequently, it was necessary to work during the past season upon the insect which we considered as second choice, i. e., *Stomoxys calcitrans*. This fly is present everywhere in the state from early July until cold weather, increasing as the season progresses. They are abundant about the University Farm, and altho swamp fever cases have been kept there for several years in stables and in pastures no case of transmission has occurred which could by any manner of reasoning be laid at the door of *Stomoxys*. As stated previously, if it was the common carrier of the disease, widespread epidemics might be expected to occur.

Stomoxys was bred in large quantities until a dry period came on in late July lasting thru August. The breeding jars were quickly depleted by this sudden excessive heat and wild flies began to become scarce also. For this reason the last lot of flies used were wild flies captured in the dairy barn.

The first attempts to feed *Stomoxys* by hand were so discouraging that this method was abandoned. They refused to feed when placed in large test tubes, lamp chimneys and wire gauze cylinders; but as soon as let free in the cage went to the animal at once and fed eagerly. Flies were, therefore, liberated in the large cage and allowed to feed at will. Two phases of the experiment were run simultaneously. In the small isolated cage the horse acting as reservoir of the virus and the healthy horse were alternated, leaving each one in for one hour until

the flies had been able to feed, next morning the other horse was left for an hour, etc. This cage was called Cage A.

In Cage B, where the horses were kept separate by a large mesh double wire partition, the flies were allowed to feed at will. The same horses were used in this cage, being returned there each morning after the hour in Cage A. When a fresh lot of flies was let into this cage the horses were tied close to the double partition and two attendants gently brushed them back and forth thru this partition so as to make doubly sure of the possibility of mechanical transmission. During the last few days of feeding the flies the horses were tied close together in one cage, their heads being carefully covered and other precautions being taken to prevent possibility of actual contact and flies fed and brushed back and forth from one horse to the other until they had become full fed.

Horse 25 was again used a source of virus, as his blood had been proved virulent during the previous winter by subinoculations. For part of the experiment Horse 36 was also used. This was a virulent case brought in the previous September. During the course of the experiment he succumbed to the disease. Horse 38 was the healthy horse.

The feeding of the flies began on July 20 and ended August 31. In Cage A, where it was hoped opportunity would be given for biological transmission, a total of 1,425 flies were used. In Cage B, the double cage, 2,800 flies were used. The flies invariably fed well, but could not be made to survive more than about one week on the average, altho every detail to aid their survival was supplied. Probably the unusual hot, dry weather hastened their death, as it was doing with the wild flies.

Horse 38 remained normal until October 14, when it fell and dislocated its shoulder, making it necessary to kill it. The incubation period in a case like this might last for three months so that the results here are not as definite as were desired. Blood was taken from the horse before death, however, and inoculated into Horse 42. Horse 42 showed definite febrile reactions on November 6 and 7, December 10 and 11 and December 17, 18 and 19; the high temperature continuing more or less marked from the last date until its death on January 11, 1917.

Blood from Horse 42 was filtered and injected into Horse 43 on February 24. This horse showed typical febrile reactions on March 10 and 11, April 10, 11 and 12, April 29 and 30, May 1 and 2, May 23 to 26. On the last date the horse was down and was killed.

From this experiment it seems probable that swamp fever of horses can be carried from one horse to another by the biting stable fly, *Stomoxys calcitrans*.

The results of these experiments, together with a study of the investigations of other workers has not, however, fully convinced us that insects are the usual or only carriers of the disease.

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THE INTESTINAL WORMS OF DOGS IN THE PHILIPPINE ISLANDS

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During the Spring of 1916 I was able, through the kindness of Professor Daniel de la Paz of the Department of Pharmacology of the College of Medicine and Surgery at Manila, to examine the intestinal tracts of 118 Philippine dogs. These dogs had been brought to the laboratory for teaching purposes and had been killed before recovery from the anesthetic. As there has never been any report made on the intestinal worms of dogs in the Philippine Islands, and as many of the findings were interesting and unusual, I feel that I am justified in reporting my findings.

The dogs which were examined were practically all collected by the city dog catchers and brought to the medical school from the city pound. At the pound, the dogs are kept in large cages with concrete floors which are daily washed and disinfected. There is a cage for each day of the week and when the dogs have been kept for seven days those which have not been claimed by their owners are either electrocuted or sent to the medical school for experimental and teaching purposes. As the work of the dog catchers extends all over the city of Manila, it will be seen that this series represents the average of the unclaimed dog population of the city. They are the canine nomads which forage about on the streets, and of which only a very small proportion ever fall into the hands of the dog catchers.

In making the examinations, the esophagus, stomach, and all of the intestines were removed from the body and opened in water. After thorough irrigation of these viscera, the worms which remained attached to the walls of the organs were removed and the washings were carefully examined for loose worms. The liver and bile ducts of about half the subjects were examined for trematodes, but none was found. The following table shows graphically the prevalence of infections.

	Number	Per Cent. Infected
Dogs examined	118	
Dogs infected	115	97.45
Hookworms (<i>Ancylostoma caninum</i>)	114	96.61
<i>Toxascaris limbata</i>	8	6.77
<i>Gnathostoma spinigerum</i>	8	6.77
<i>Spiroptera sanguinolenta</i>	7	5.92
<i>Dipylidium caninum</i>	55	46.56
<i>Dibothriocephalus</i> sp.	7	5.92
Total infections	199	168.55

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There were 52 dogs, or 44 per cent., in which two species of worms were found as follows:

Hookworm and Dipylidium, 43; hookworm and Dibothriocephalus, 3; hookworm and Spiroptera, 3; hookworm and Ascaris, 2; hookworm and Gnathostoma, 1.

There were triple infections in 12 dogs, or 10 per cent. These were the following:

Hookworm, Ascaris and Dipylidium, 3; hookworm, Ascaris and Dibothriocephalus, 1; hookworm, Dipylidium and Dibothriocephalus, 2; hookworm, Dipylidium and Gnathostoma, 3; hookworm, Spiroptera and Gnathostoma, 3.

One dog was infected with 4 species: hookworm, Ascaris, Dipylidium and Dibothriocephalus, and one with 5 species: hookworm, Ascaris, Dipylidium, Gnathostoma and Spiroptera.

Hookworms — 97.45 per cent.

All the hookworms which I examined belonged to the species *Ancylostoma caninum* (Ercolani, 1859). The number of worms present in an individual was found to vary from 2 up to 300 or 400. As well as being the most common this is probably also the most harmful worm parasite of dogs in the Philippine Islands. It is the cause of death of a large percentage of young dogs, particularly those of the better breeds and those which have been imported. The common street dogs seem to be more or less immune to the effects of this parasite, but even they are always emaciated and anemic when the worms are present in any considerable number. This hookworm is also very common in cats around Manila.

Toxascaris limbata (Railliet et Henry, 1911)—6.77 per cent.

The percentage of infections with this form was much lower than I had expected to find it, and the number of worms present in each case was very small. The fact that the majority of the dogs examined were full grown may account for the small percentage shown. A veterinary surgeon informs me that this parasite is very frequently found in puppies here in Manila, while they are only rarely encountered in older dogs.

Gnathostoma spinigerum (Owen, 1836) — 6.77 per cent.

Eight dogs were infected with this worm which has been reported from dogs in India by Mitter. The worms live in large cysts in the wall of the stomach which are connected with the lumen of that organ by a small pore. Cysts may contain anywhere from 1 to 11 or 12 worms, and there may be 3 or 4 cysts present in a stomach. In one case I found *Gnathostoma* and *Spiroptera* present in the same cyst. This form is also frequently found in cats.

Spiroptera sanguinolenta (Rudolphi, 1819)— 5.92 per cent.

This worm is also found in cysts in the stomach and frequently in the lower part of the esophagus. In one dog there were three cysts in the esophagus and two in the stomach which yielded in all 18 worms. The cysts of *Spiroptera* and *Gnathostoma* present the same gross appearance and it is necessary to cut into them in order to determine which worms are present.

Dipylidium caninum (Linneus, 1767)—46.56 per cent.

This tapeworm was found in 55 out of the 118 dogs examined. In the majority of cases the number found ranged from 1 to 10 specimens, but in some instances they were very numerous. In such cases the majority of the worms were young and small. In one dog there were 2 specimens each about 20 cm. long, with ripe proglottids, and in addition I picked off from the surface of the intestine 196 individuals with heads the largest of which did not exceed 5 cm. in length.

Dibothriocephalus sp.

This is a small species of *Dibothriocephalus* which is more common in cats than in dogs in the Philippines. The entire worms measure from 40 to 62 cm. in length, and the largest segments at the posterior end are about 7 mm. wide by 2 to 3 mm. in length. The head closely resembles the head of *Dibothriocephalus latus*. I have never found more than three specimens in a single host. Specimens of this worm have been sent to Dr. H. B. Ward who has kindly consented to identify them.

The total absence of any tapeworms of the family Taeniidae from this series proved a surprise to me, and I might add that I have never found a *Taenia* in either a dog or a cat in the islands. Several cases of larval infections of man with *Echinococcus granulosus* have been reported, but I have not been able to find any authentic report of the finding of the adult in the islands. The absence of some of the other *Taenia* forms which are found in dogs may be accounted for by the fact that suitable intermediate hosts—rabbits and sheep—are not found in the islands.

THE INTRACELLULAR DEVELOPMENT OF A GREGARINE *FRENZELINA AMPELISCA* N. SP.

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The parasites described below are found in the small Amphipod, *Ampelisca spinipes*, collected from the Eel Pond, Woods Hole, Mass., during August, 1912, and April, May, and August, 1914. Examinations of hosts were made at the dates mentioned through a series of more than two hundred carefully sectioned and stained slides, as well as on the living material, and few cysts were seen. That we have not yet found the season for their production is possible, but what is more probable is that only the vegetative stage is passed in *Ampelisca* and the spore stages in another host or free in the water. With this idea we have decided to publish what we have in the hope that someone may later complete the life-history.

The best fixation was found to be in Schaudinn's fluid, though Bouin's and sublimate-acetic were used satisfactorily. Sagittal sections, ranging in thickness from 7 to 25 μ were stained with either Heidenhain's iron-hematoxylin or Mann's methylene blue-eosin mixture. Both methods gave good results.

The only part of the anatomy of *Ampelisca* which interests us here is that of the digestive tract and its glands. The alimentary canal consists of a straight tube from mouth to anus, divided into three general parts, stomadeum, mid-gut and proctodeum. The stomadeum and proctodeum, both of which are relatively short, are lined with a chitinous material, which in the stomadeum forms the masticatory stomach. The long, straight mid-gut makes up the principal part of the digestive tract. At its anterior and posterior ends it is lined with large epithelial cells of the columnar type, while in the central portion the epithelial cells are much more flattened. A very thin, delicate, non-chitinous cuticle, described in certain of the amphipods, is present over the digestive epithelium of *Ampelisca*.

The hepatic ceca, four in number, empty into the anterior end of the mid-gut. They are very long, two of them extending almost to the posterior end of the animal. The cells making up the hepatic ceca are large with characteristic vacuoles and large nuclei having karyosome-like nucleoli.

There are also a pair of small, pouch-like glands at the posterior end of the mid-gut and a single short dorsal cecum anterior to the junction of the hepatic ceca with the mid-gut.

Frenzelina ampelisca nov. spec.

The earliest stages of this parasite are intracellular, occurring in the digestive epithelium throughout the entire length of the mid-gut. In one host these very small intracellular forms were extremely numerous from the junction of the stomodeum with the mid-gut to the proctodeum, all stages of their growth being found. As there is often a pure infection of this form there is no danger of confusing it with two other gregarine inhabitants of *Ampelisca*.

Figures 1 and 2 represent two of these earliest forms, smaller than the nuclei of the epithelial cells. There is not a great deal of structure to be seen in them at this time. The protoplasm is more deeply stained and much less vacuolar than in the older stages. The nucleus is characteristically vesicular with a large, dark karyosome. No division of the body into protomerite and deutomerite is yet to be seen. The form represented in Figure 1 is 3.75 by 2.5μ .

The parasite is distinctly like the older form when it attains a size of 13.75μ by 5.55μ (Fig. 3). At this time it is usually very vacuolar, the cytoplasm having the appearance of a coarse reticulum. In this stage the body is always divided distinctly into protomerite and deutomerite, the protomerite being thick and short, from one-fifth to one-third the length of deutomerite. The cuticle is very thin and shows no signs of either cross or longitudinal striations or of myonemes. The nucleus is vesicular in form with a large, deeply staining karyosome, a distinct membrane and a homogeneous space between karyosome and membrane. A second small nucleus of the vesicular type with a deeply staining karyosome can usually be distinguished in the protomerite, even in very young forms (Figs. 4 and 6), occasionally a few unorganized chromatin granules are found in its place, and there are a few cases in which it seems actually to be lacking. Léger and Duboscq (1907) have described such a nucleus, which seems to be transitory and takes no part in reproduction in *Pterocephalus*.

The nucleus of the host cell is usually displaced by the young gregarine (Figs. 3 and 4), and as growth proceeds the parasite pushes out until the cell in which it was originally clearly located can no longer be distinguished. The gregarine makes for itself a considerable cavity among the epithelial cells (Figs. 5 and 7).

The nuclear structure of the parasite remains the same except for a proportional growth with the cytoplasm, but, on the other hand, the cytoplasm of the larger intracellular forms shows a marked change from that of the younger condition. This change is not always constant, however, for a given size of the parasite.

Figure 7 represents the largest intracellular form found, being 60.62 by 13.12μ . This form shows quite distinctly the change in cytoplasm,

which has become filled with large, clear granules instead of being reticular as in the early stage. These granules may be of the nature of stored food material for the reproductive process.

Figure 15 shows the not uncommon occurrence of this parasite in the cells of the hepatic ceca. Stages as small as those sometimes seen in the intestinal epithelium have not been found in the liver, nor have the large forms with the granular cytoplasm been found there. Figure 15 is a good example of the type found in the cells of the hepatic ceca. The protomerite and its nucleus are distinct as in the intestinal forms, and in every essential these gregarines resemble the intracellular stage in the intestinal epithelium with the exception of a greater tendency for the protomerite to be subdivided either once or twice (Figs. 15 and 16) giving a papillate appearance.

A few forms have been found free in the lumen of the mid-gut. With two exceptions these resemble the intracellular parasites of the same size, sometimes with a subdivision of the protomerite, a flattened knob, sometimes without.

Figure 8 represents one of the exceptions. In this form the cuticle presents a thickened appearance and is distinctly grooved longitudinally. The protomerite shows an indistinct division, beyond which the grooves do not extend. The cytoplasm is of the characteristic, mottled, reticulate type, and the nucleus typically vesicular but with four small patches of chromatin closely applied, at different points, to the membrane. There are two nuclear bodies in the protomerite of this specimen.

The other exception is a form similar to the one just described, but with the cuticle much thicker and quite shining. The grooves are also more conspicuous. The whole thing stains deeply and is too indistinct to show structural details.

A few free forms, similar in all respects to those found within the cells of the hepatic ceca, were found in the lumen of those glands. Figure 16 represents the biggest, thickest form found in the hepatic ceca. It is 43.75 by 18.75μ and is partly embedded at the protomerite end, in the cells of the gland. It differs from most forms by showing a deeply staining mass of granules surrounding the nucleus. Extracellular forms of this parasite are rare.

Young gregarines, no doubt, pass from the digestive tract into the hepatic ceca at the point where they join the mid-gut. They seem also to pass directly through the walls of the intestine and glands, into the coelom. Circumstantial evidence points to the latter as being at least one method by which exit from the intestine may take place. A number of cases of the intracellular forms in the intestine breaking through on the coelomic side of the wall have been observed. Figures 5 and 6 represent two forms, one just beneath the membrane on the coelomic

side of the intestinal wall, and the other apparently pushing its way out. These forms were found at the extreme anterior end of the mid-gut where the epithelium is quite thick. Still other forms were found more than half way through the wall in the central region of the mid-gut.

Only two examples of what is probably copulation were found (Figs. 11 and 12). This process takes place end to end. The sporonts (Fig. 11) are attached to the digestive epithelium at the anterior end of the mid-gut. This stage must follow very quickly upon that of the largest intracellular form (Fig. 7), for they differ in no essential detail from that form. The cytoplasm is filled with large, clear granules, and the nuclei are typically vesicular with a large, deeply staining karyosome.

Union takes place by the pushing of the protomerite of one form in the posterior end of the other. The outline of the protomerite containing a faint but evident second nucleus can be plainly seen within the body of the satellite. This copula is attached to the epithelial cells near the anterior end of the mid-gut. The method by which the sporonts join each other seems to be a sucker-like invagination on the posterior end of the primite. That there may be such an invagination before the two are united is shown in Figure 16.

A later stage of copulation was also found, and is represented by Figure 12. This pair occurred free in the lumen of the mid-gut. The difference in size of the sporonts is very noticeable, the primite measuring 62.5 by 15.62μ , the satellite 31.25 by 6.25μ . The protomerite of the satellite fits into an invagination of the posterior end of the primite. The protomerite is modified in each sporont and its nuclear body has lost the membrane, but the chromatin mass may be seen in each. Likewise the form of the deutomerite is changed from the earlier copula as seen in Figure 12. The cytoplasm contains many coarse granules and stains very deeply. The primite is much swollen and is vacuolar.

Léger and Duboscq (1909) found similar copula in *Frenzelina conformis* and designated them as old copulating pairs ready to form cysts. One cyst was found in the lumen of the intestine showing unequal sporonts (Fig. 12), but we cannot say certainly that it belonged to this parasite. Whether the cyst formation takes place in the intestine of this host can probably be determined by a study of *Ampelisca* later in the fall and winter. We have investigated them in spring and found none, infection even in the early trophozoite stage being slight at this time. Because cysts containing two copulants were found free in the water containing *Ampelisca*, and because so few old copula are found in the intestine we suspect that *Frenzelina ampelisca* is often shed with the feces during the copula stage and encysts in the water.

SUMMARY

A new species called *ampelisca* is added to the genus *Frenzelina*, created by Léger and Duboscq (1907) for certain crustacean gregarines.

The distinguishing character of this species are (1) smaller size than other species reported and (2) possession of a nucleus in the protomerite.

This is the second time intracellular development has been reported for *Frenzelina*, Watson's (1916) being the first report.

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EXPLANATION OF PLATE

FRENZELINA AMPELISCA

All drawings made to scale with Spencer 8 oc. and $\frac{1}{12}$ oil immersion objective, except two diagrams, Figures 18 and 19.

Figures 1 to 13, show development of the parasite beginning with the earliest stage found in the intestinal epithelium.

Fig. 1.—Earliest intracellular stage found. 3.75 by 2.5 μ .

Fig. 2.—Slightly larger stage. Still smaller than epithelial nucleus.

Fig. 3.—Young trophozoite growing inside epithelial cell of structure wall; shows protomerite; cytoplasm very vacuolar. 13.75 by 5.55 μ .

Fig. 4.—Intracellular forms found near posterior end of intestine. Protomerite of one showing two nuclei; this sporont 15.61 by 6.25 μ . Lower sporont 17.5 by 5.62 μ .

Fig. 5.—Looking squarely on a form embedded in intestinal epithelium. Showing distinct nucleus in protomerite. Cytoplasm reticular. 28.75 by 9.1 μ .

Fig. 6.—Apparently going through intestinal wall into colome. Upper 23.12 by 7.5 μ ; lower 20 by 8.12 μ .

Fig. 7.—Largest intracellular form found, 60.62 by 13.12 μ .

Fig. 8.—Free in lumen of intestine. Shows thick cuticle with definite longitudinal constrictions. Also two nuclei in protomerite. 42.5 by 11.25 μ .

Fig. 9.—Free intestinal form showing no cuticle, 35.62 by 12.5 μ .

Fig. 10.—Inside intestine lying close to epithelium. 30 by 5 μ .

Fig. 11.—Conjugating pair attached to epithelial lining of intestine. Primate 56.25 by 10.6 μ . Satellite 56.25 by 8.7 μ .

Fig. 12.—Old pair of conjugants. Primate 62.5 by 15.62 μ . Satellite 31.25 by 6.25 μ .

Fig. 13.—Cyst found in intestine showing two sizes in the sporonts.

Fig. 14.—Sporozoites in intestinal lumen.

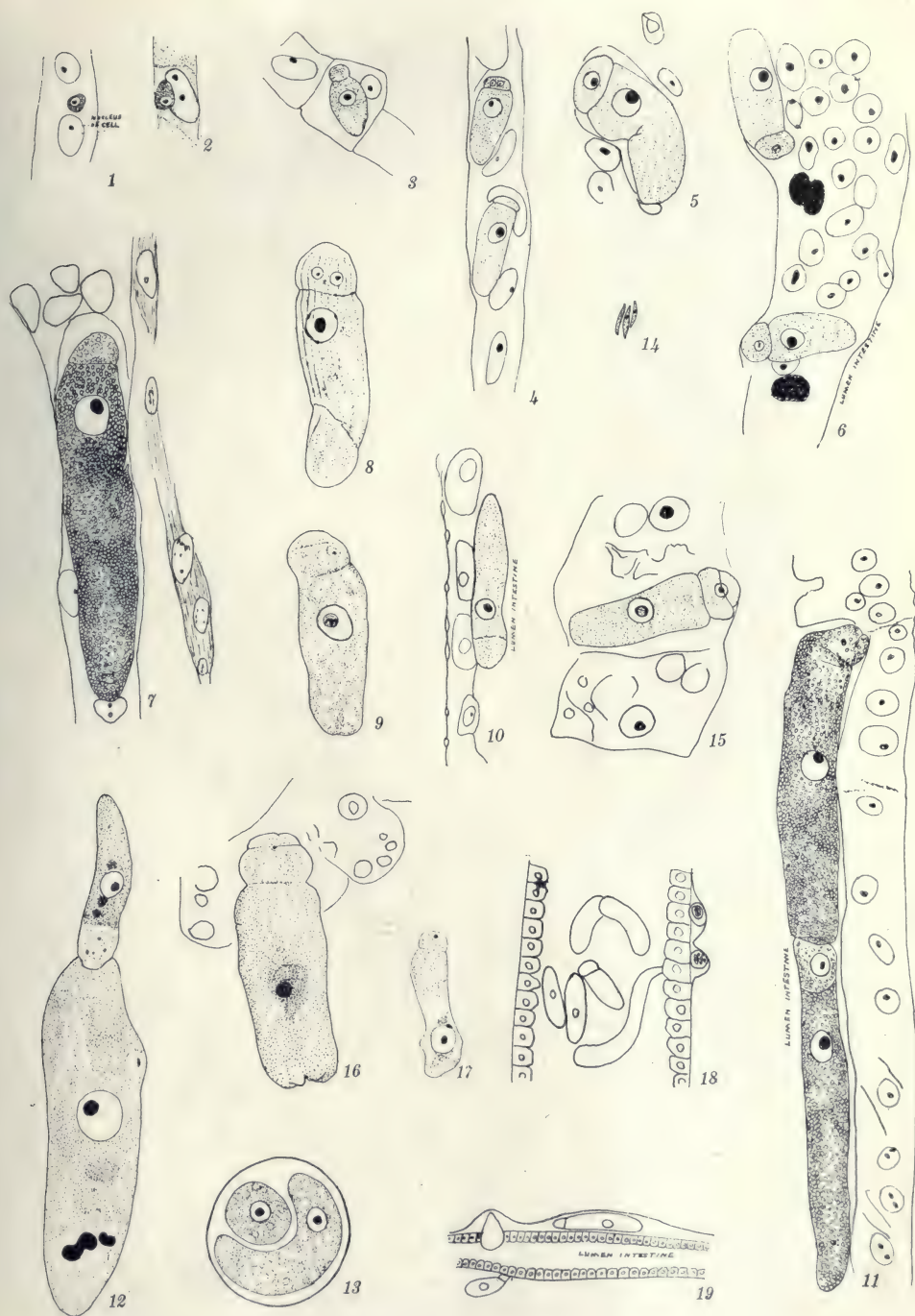
Fig. 15.—Young gregarine in liver tubule cell. 31.25 by 8.75 μ .

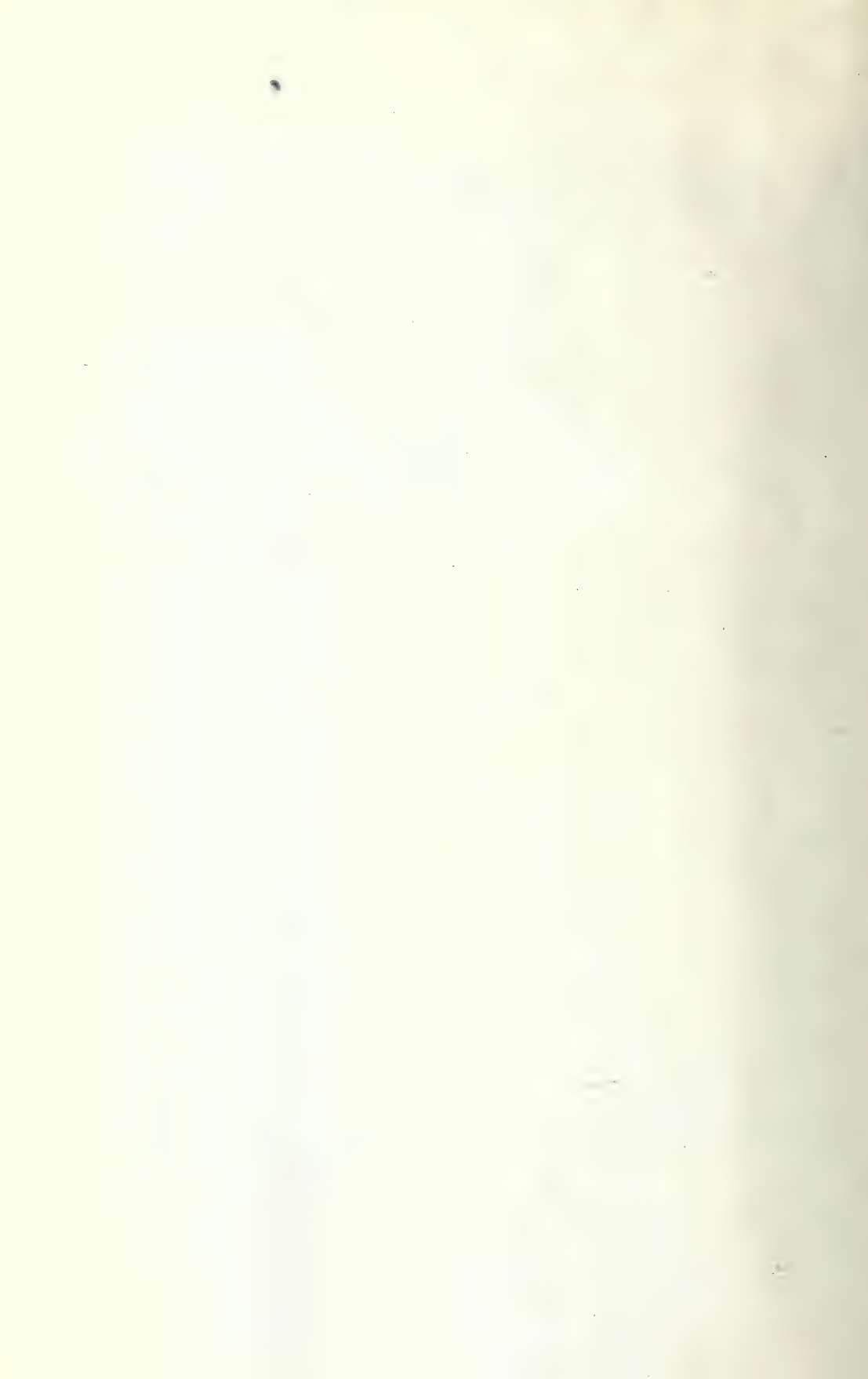
Fig. 16.—Form protruding posterior end from a cell in hepatic cecum. Protomerite is constricted into a sort of epimerite. Nucleus is extruding chromatin. Posterior end of dentomerite invaginated. 43.75 by 18.75 μ .

Fig. 17.—Shows chromatin extrusion. 25 by 6.25 μ .

Fig. 18.—Diagrammatic sketch from living gregarines showing what is believed to be this form loose in the intestinal tract.

Fig. 19.—Diagrammatic sketch from living material showing the penetration of the intestinal epithelium by one gregarine, and the resting position of another just beneath the submucosa.





ANIMAL PARASITES OF RATS AT MADISON, WISCONSIN

ARTHUR M. MOLL

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According to Blandford, the so-called brown rat, *Mus norvegicus*, came originally from Central Asia, but has spread until it inhabits practically every civilized portion of the world with the exception of South America. This rat is extremely savage and very cunning, adapting itself readily to meet different conditions of life. It seems to thrive in all climates, and is found in Iceland as well as in India. The observations to be noted in this paper were limited to the examination of twenty-five rats captured in Madison, Wisconsin, for the purpose of learning what parasites they were carrying.

Of the rats examined, seven came from a fraternity house, fifteen from a dump in the Italian district, and three were trapped on a dump near the University. All carried parasites: thirteen (53%), fleas; fifteen (60%), lice; three (12%), mites; twenty-two (88%), intestinal round worms; one (4%), trichina, and five (20%), the dwarf tapeworm. I was unable to find any blood parasites in the rats examined, but I have seen slides of *Trypanosoma lewisi* from rats captured in Madison by Dr. William Middleton in 1916.

LIST OF PARASITES FOUND

Haematopinus spinulosus Burm. Rat Louse

This louse is common on rats and has not been reported from other hosts. It transmits an apparently non-pathogenic protozoan parasite, *Trypanosoma lewisi*, from one rat to another.

Ctenocephalus canis Curtis. Dog Flea

This is the most common flea infesting houses in the eastern United States. It attacks man and most of the domestic animals. It is readily transmitted from one locality to another through the agency of rats. As it attacks both rodents and man it can readily become a transmitter of the plague. It was shown by Grassi that this flea could be the intermediate host of the tapeworm *Dipylidium caninum*.

Ceratophyllus fasciatus Bosc. Rat Flea

This is strictly a rat flea in that it requires rat blood as a food in order to reproduce. However, it was found by Strickland that while this flea completes its life cycle only on the rat, it bites man in preference to rats when given the choice. This fact makes it an important factor in plague-infected localities.

Laelaps agilis Koch. Mite

This mite is found commonly on the rat in small numbers. It is apparently of little economic importance, not commonly attaching itself to man or to domestic animals.

Trichinella spiralis Owen. Trichina

From an economic standpoint this worm is without doubt the most important parasite carried by the rat, at least in the United States where plague is not prevalent. Trichina is conveyed from rat to pig, and, by eating uncooked or imperfectly cooked pork, from pig to man. It sometimes causes severe and very fatal epidemics and necessitates large expenditures for meat inspection.

Heterakis spumosa Schneider.* Roundworm

This nematode is common in the cecum of the rat and is of world-wide distribution. It is of practically no economic importance being exclusively a parasite of the rat and apparently not affecting it to any extent. I examined rats which appeared to be enjoying the best of health and which had hundreds of these worms packed in the cecum.

Hymenoleps diminuta Rudolphi. Dwarf Tapeworm

The cystocercoid of the dwarf tapeworm develops in the body cavity of a number of meal-infesting insects. Grassi believes that the caterpillars of Lepidoptera are the usual intermediate hosts. The insects take up the eggs scattered by rats. It has been experimentally demonstrated that men may develop this tapeworm by swallowing infected insects. According to various texts, natural infection probably occurs by ingesting such insects with cereals or imperfectly cooked foods.

Through its migratory habits, its varied diet, and its intimate association with man, the rat is of considerable importance as a carrier and transmitter of parasites. The investigation in this instance has been rather limited. A survey of the literature shows that over eighty species of parasites have been reported from a number of different localities and are apparently common to the rat. All the parasites found in the rats captured in Madison have been reported as occurring in other localities. The finding of trichina in this neighborhood emphasizes the importance of a rigid inspection of the pork. The species of fleas found on the rats of Madison are all capable of transmitting *Bacillus pestis* and might be of importance in case of a plague epidemic.

REFERENCE

- Shipley, A. E. 1908. Rats and Their Animal Parasites. Jour. Econ. Biol., 3: 61-83; 4: 19.

* For the identification of this nematode I am indebted to Dr. B. H. Ransom.

NOTES

To the Editor: In *The Lancet* (London) for Dec. 25, 1915, No. 4817, there appeared an article in which I stated, "Early in June he (i. e., Dr. E. Warren) notified me that he had found sporocysts containing cercariae with bifid tails in the liver of one of these snails. . . . The appearance of these cercariae (for which I suggested the name of *C. secobii*, in memory of our old school in Kent) was similar to that described by Leiper and Atkinson. The cercaria consisted of a body containing two suckers, one oral and one ventral; no pharynx could be detected and there were no pigment spots. The tail of the cercaria was bifid for half its length and showed no indication of cuticle covering."

In the *Medical Journal of South Africa*, June, 1916, vol. 11, referring to the same cercaria, I said: "Another common form, for which I have suggested the name of *Cercaria secobii*, has very long prongs to the tail, sometimes even longer than the tail itself. I have found it only in specimens of *Physopsis* collected from the Umsindusi River."

In THE JOURNAL OF PARASITOLOGY for March, 1917, v. 3, no. 3, I described this cercaria as *Cercaria secobiana*; but, in view of the references to it in journals of an earlier date, the name *Cercaria secobii* has priority.

Yours, etc., F. G. CAWSTON.

To the Editor: May I make a suggestion to the men who are doing work of a nonsystematic character with different lower animals? When a man writes on some question relating to genetics, or embryology, or phototropism, or ecology, or what not, he should give the name of the specialist who named the species he is considering. This point will readily appeal to you. If I were to write a paper in which the name of a beetle was given, my accuracy would be attested by the fact that I inserted in parenthesis "Determined by Schwarz"; or, if it were a Protozoan, the same thing would happen if I inserted in parenthesis "Determined by Calkins." With a little care in this way subsequent workers would not be put to the trouble to see whether the man was writing about the form he said he was.

(Signed) L. O. HOWARD.

In Japanese Medical Literature (vol. 2, no. 5) is reviewed the work of M. Muto on the life history of *Metagonimus yokagawai* found in Japan chiefly but also in Formosa and among Japanese in Korea. The rediae and cercariae were discovered in *Melania livertina*, also incriminated in connection with *Paragonimus westermanni*, and *Clonorchis sinensis*. The cercaria is long-tailed, provided with 4 to 6 oval spines and with two eye spots. It measures 0.23 by 0.083 mm. and the tail 0.29 by 0.027 mm.

Uninfected dace and gold fish were kept in water containing free cercariae from these snails and after a time encysted cercariae were found under the scales, in the tail, or at the base of the fins. Infected fish tissue was fed to dogs and in 12 days eggs of the fluke appeared in the feces of the dog.

Marked differences in the size and shape of cysts from various regions seemed to the reviewer to suggest possible confusion of different species. Kittens were invariably infected within 12 to 15 days after eating infected fish.

BOOK REVIEWS

THE MICROSCOPE. Simon Henry Gage. An Introduction to Microscopic Methods and to Histology. Ithaca: The Comstock Publishing Company, 1917. ix + 469 pages. 252 figures. \$3.

This, which is the twelfth edition of the well-known work, has been considerably rewritten and stands as a splendid testimonial of the continued scientific activity of the author. Those who recall the earlier editions would hardly recognize the work in its present form. It has reached the stately dimensions of 472 pages, with 252 illustrations. Some use has been made of fine type, so that there is a large amount of information compressed into the limits of the work. The analytical arrangement of facts makes it a work of great value for the biologic student who is seeking to gain a thorough command of the microscope and the technic of its application to biologic research in any direction.

THE DIAGNOSTICS AND TREATMENT OF TROPICAL DISEASES. E. R. Stitt, Medical Director, U. S. Navy. Second Edition, Revised and Enlarged. Philadelphia: P. Blakiston's Son and Company, 1917. xiii-534 pages, 117 figures. \$2.00 net.

As stated in the review of the first edition of this work in *THE JOURNAL* it deserves especial commendation since the author is a parasitologist and speaks with authority of the animal organisms concerned. While in general appearance this is like the earlier edition it deserves to be listed as a new work because of the recent material added. In bulk it has received additions equaling one fourth of the original and the illustrations are even more greatly increased.

The changes which have been made are first of all in Part I two new chapters, one dealing with typhus fever and a second on tick fever, better spotted fever of the Rocky Mountains, as the author calls it. In addition new material has been added to various topics and other sections have been entirely rewritten. The use of different types has been resorted to in order to assist the busy worker, a procedure which has lost some part of its value by lack of consistency in its application.

Part II, the section on diagnostics, has been modified more than Part I, because, as the author says, "one can more readily acquire skill in the differentiation of diseases by considering them when grouped according to clinical manifestations than when treated separately." These changes are undoubtedly of great value and it is unfortunate that the author has retained the inadequate figures of the ova of parasitic worms; these are photographs of charts that, while good in the original perhaps, are really of little value to the student with the microscope since they present a picture of the real objects that will not serve for diagnostic purposes.

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STUDIES ON ILLINOIS CERCARIAE *

ERNEST CARROLL FAUST

For two and a half years the writer has been studying the cercariae of Illinois. Material has been secured from the Rock River drainage at DeKalb and Mt. Morris, and from the Sangamon drainage at Urbana and Homer. In all cases the larvae were found in the common snails of the area, *Planorbis trivolvis*, *Physa gyrina* and *Goniobasis pulchella*.

The snails were dissected and the infected organs teased out in one-half normal saline solution. This solution was found to be quite satisfactory as an isotonic medium, although different species of fluke larvae vary considerably in their osmotic equilibria. In all cases in the present paper the exact course of the excretory capillaries has been traced and in six species the minute structure of the flame cells has been studied. A portion of each infected tissue has been preserved. Gilson's fluid has been used as a fixing agent. Toto mounts of the flukes and sections of the infected tissues have been studied to supplement the work on the living material. The former have been stained in a dye consisting of one part each of stock Delafield's hematoxylin and Ehrlich's acid hematoxylin in twenty-five parts of an aqueous solution of saturated ammonium alum. Sections have been stained with Delafield's hematoxylin with an eosin counterstain. The preserved material has been most valuable in observing the genital organs and the histological structure of the worm.

In a previous study the writer (1918) has shown that the infection of the mollusks of the Bitter Root Valley, Montana, varies within very limited areas but that the same larvae are found in the valley from one season to another. The Illinois cercariae are much more variable both in seasonal and locational distribution, but are on the whole more cosmopolitan species. *Cercaria trivolvis* and *C. isocotylea*, described by Cort for Urbana in 1913, were found by the writer in the Normal School pond at DeKalb in August, 1917. A furcocercous form, *Cercaria gigas*, secured from *Planorbis trivolvis* at DeKalb and

* Contributions from the Zoological Laboratory of the University of Illinois, No. 105.

from *Physa gyrina* in Pine Creek of Rock River in August, 1917, was not present in the DeKalb mollusca in October, 1917, but was found in *Planorbis trivolvis* at Urbana during this month, although not previously reported from that area. On the other hand, the finding of certain monostome cercariae (*Cercaria robusta* and *C. aurita*) only in a single locality does not necessarily argue for the limitation of these species to that area alone.

Habits and morphologic features of cercariae have been studied in isolated instances. Significant investigations of recent years have been made by Ssinitzin (1905, 1911), Cort (1915), and Faust (1918). Of the fifteen species described by Cort, ten were found within the state of Illinois. Of these, four were taken from Urbana, four from Chicago, and one each from Rockford and Mahomet. One species (*C. inhabilis*) was found in *Planorbis trivolvis* at Urbana and also at Lawrence, Kansas, (Cort, 1915), one (*C. douthitti*) at Chicago and Douglas Lake, Michigan, (Cort, 1917), and another (*C. diastrophia*) at Chicago and Lawrence, Kansas (O'Roke, 1917). The present study includes records of two species (*C. isocotylea* Cort and *C. trivolvis* Cort) from both Urbana and DeKalb, and *C. gigas* from Urbana, DeKalb and Pine Creek.

MONOSTOME CERCARIAE

Cercaria robusta nov. spec. (Figs. 1-5)

Host: *Physa gyrina*.

Locality: Normal School pond, DeKalb.

Collected in August and October, 1917.

Parthenita: redia.

The worm for which the name *Cercaria robusta* is proposed is broadly spatulate, more or less acutely ovoid anteriorly and obtusely rounded posteriorly. The tail is extremely muscular, hence capable of great contraction, and is much shorter than the body. The length of an average mature specimen is 0.32 mm. and the width is 0.15 mm. The tail is about 0.15 mm. long and 0.06 mm. wide at its proximal end. There is a pair of lateral eye-spots and a single median eye on the dorsal surface just behind the pharynx. Melanoidin granules are imbedded in the hypodermal tissues over the central nervous system and extend posteriorly along six lines, two dorsal, two lateral, and two ventral, marking out superficially the main posterior nerve trunks (Faust, 1918). At the postero-lateral margins of the animal are a pair of locomotor pockets, each of which is provided with a few large gland cells at its inner end (Fig. 1).

The redia averages about 2 mm. in length by about 0.4 mm. in width. Its pharynx is small but powerful. The rhabdocoel gut is longer than the cavity of the redia and is coiled forward in the region

of germ-ball proliferation. The posterior end of the redia is frequently top-shaped. The germinal epithelium lies in this posterior tip, from which the germ balls are derived. All of the rediae observed were producing cercariae. Neither lateral feet nor collar nor birth pore have been observed in the redia of this species.

The excretory system opens dorsad from an oval bladder through a small pore. Two main trunks arise from a common head just over the posterior portion of the pharynx. Each trunk receives a common external lateral halfway back from the anterior end of the system. The lateral is found to be derived from an anterior and a posterior longitudinal canal which run parallel to the main trunk. These external canals have their origin in very small capillaries. A single flame cell is probably at the head of each capillary (Fig. 1).

From an ovate pharynx 38μ in diameter the digestive tract leads back through a short esophagus to a pair of furcae which reach to the subcaudal region of the body. The furcae are not conspicuous in the living animal and are made out with difficulty in the toto mounts but may be observed in sections.

The nervous system of the animal is outlined superficially by the pattern of the melanoidin granules. The main cerebral mass is dorsal to the esophagus, forming a saddle over that organ. The pair of ventral trunks is the most conspicuous of the anterior series, while all three trunks of the posterior series, dorsals, laterals, and ventrals, are equally well developed. Transverse commissures between posterior trunks are frequent. The pair of eye-spots is lateral to the ganglion, center on the dorsal side, just anterior to the junction of the dorsal and lateral nerves. These eye-spots arise from the posterior dorsal trunks. The median eye-spot is smaller and the granules are less definitely massed than those in the paired eye-spots. This cyclopean eye is immediately in front of the cerebral mass. The eye structure is similar to that of *Cercaria pellucida* (Faust, 1918).

The genital organs are clearly outlined in *Cercaria robusta*. The ovarian cell mass is skull-cap shaped; it lies just in front of the excretory bladder. A uterine duct is represented by a chain of cells which arises just anterior to the ovary and ends in the anterior third of the worm, a short distance behind the lateral eyes. A small cell mass at its anterior end is the vagina. The two small testes are to the right and left of the excretory bladder. The vasa efferentia arise from these cell masses and, bending around the ovary, unite just anterior to this organ to form the vas deferens. The vas deferens lies to the left of the uterus and parallels it to the region of the vagina, where it ends in a small swollen mass, the cirrus. The vitelline glands are aciculate in outline. They are composed of three pairs of glands

in the outer series and four pairs and a double median gland in the inner series. The median gland is the anteriormost of the inner series and represents a fused pair. Aside from this single modification the glands are similar in position and number to those described by the writer for *Cercaria pellucida*, *C. konadensis*, and *C. urbanensis*.

The conspicuous structures of the living *C. robusta* are the longitudinal muscle fibers of the tail. More deeply located in this organ are six pairs of large gland cells. These correspond in grouping to the six pairs of gland cell groups in the tail of *C. konadensis* and are identical in number and structure to the six pairs of gland cells in the tail of *C. urbanensis*.

The mature *Cercaria robusta* breaks through the wall of the redia and penetrates the liver tissue of the host. It may either work its way to the free water or encyst in the liver sinuses. The movement accomplished by the coordination of oral sucker and posterior locomotor pockets is slight; most of the locomotion comes from the activity of the tail. Due to its extensive muscularization, this organ acts as a powerful whip-lash, stirring up a whirlpool eddy all around it by its violent movements. When this cercaria is set free into the water it attaches itself by the oral sucker, while a whirlpool movement of the entire worm is initiated by the tail. Encystment starts immediately. Beginning at the oral sucker it proceeds rapidly backward, limiting the size of the whirlpool as encystment continues. Finally a cyst has been formed around the entire worm, while the free tail, attached to the cyst only by a fibril, continues its characteristic movement. Then the worm within the cyst twists around and loosens its connection with the fibril. For a while the tail keeps lashing after all connection with the encysted worm has been broken, but the movement of the organ tends to become less violent and at length ceases entirely.

Cercaria aurita nov. spec. (Figs. 6-8)

Host: *Goniobasis pulchella* (Anthony).

Locality: Salt Fork of Sangamon River, Homer.

Collected: October, 1917.

Parthenita: redia.

This species is designated as *Cercaria aurita* because of the lappet processes which characterize the worm just lateral to the pair of eyespots. The animal has a length of 0.57 mm. and a width of 0.19 mm. The tail is 0.33 mm. long and 0.08 mm. wide at the base. When the animal elongates the sides are parallel and the animal is roughly rectangular, save for a blunt rostrum in front of the auricular prominences. At the postero-lateral angles are a pair of locomotor pockets, which are distinctly helpful in locomotion. When the body contracts

it becomes pear-shaped. The tail is comparatively useless. The body has a dirty grayish-brown appearance.

Cercaria aurita develops within a redia about 1.5 mm. long and 0.4 mm. in diameter. The pharynx is small and leads into a large rhabdocoel gut which extends through the body cavity about three fourths the way to the posterior end. Only a few cercariae develop at any one time within the redia. They break through the heavy wall of the redia and worm their way through the water.

The excretory tract in *C. aurita* is more primitive than that of any previously described monostome cercaria. A small oval bladder at the caudal end of the body receives two dilated trunks through a common reservoir. The trunks are very short and become reduced to the size of capillaries at the loci where they turn forward. These capillaries can be traced forward for only a short distance. This reduction in the excretory system constitutes a remarkable differentiation from the circuitous system in previously described monostome cercariae. It is further distinguished by the absence of excretory granules in the canals and trunks.

The digestive tract is prominent and easily seen in the living worm. A subspherical pharynx, 13μ in diameter, leads into a long esophagus that extends through somewhat more than the anterior third of the worm. The furcae are of a length equal to the esophagus. At their blind end they are distended, so that they appear club-shaped. The cells lining the digestive tract are large and glandular.

The nervous center of the worm is diffuse. It covers considerably more ground than that of *C. robusta*. This fact is superficially recognized by the diffuse arrangement of the melanoidin granules just beneath the basal membrane of the animal. No anterior nerve trunks are prominent; all three pairs of posterior trunks are easily made out although their transverse commissures are inconspicuous. The pair of lateral eye-spots on the dorsal side is well developed. These eyes are set out some distance from the center of the body. Their connection with the brain ganglia has not been studied.

The genital organs are most unusual in their limited extent. Except for vitelline elements they are confined to the middle third of the body. The ovary is represented by a small irregular mass of cells median in position. A short string of cells, the uterine cells, leads to a spherical mass a short distance anteriorly, the vagina. The testes are slightly behind the ovary, rather irregular in appearance, and appreciably larger than the ovary. Their vasa efferentia coalesce in front of the ovary to the left of the uterus, and the common vas deferens runs forward to a spherical cirrus pouch. The vitelline glands could not be definitely made out. They are diffuse in their structure. A

small anterior portion occurs just in front of the testes. A large branch extends posteriad on the ventral side of the worm. This pattern of vitelline distribution has not been reported thus far for monostome cercariae and differs from the vitelline structure of other monostome larvae as markedly as the other features of this worm differ from those of described monostome cercariae.

The cercaria is not conspicuously active. It comes out of the mature redia as a sluggish, crawling worm. The tail is a hindrance rather than an aid in movement, for it is dragged along behind the body without any independent movement. The posterior locomotor pockets cooperate with the oral sucker in the attachment of the worm to the crawling surface. These pockets are not muscular but are provided with several large gland cells.

Encystment has not been observed, although the large number of semi-opaque cystogenous glands must function in the secretion of a cyst. The worm soon disintegrates when placed in a hypotonic medium.

ECHINOSTOME LARVAE

Cercaria chisolenata nov. spec. (Figs. 9-13)

Host: *Physa gyrina*.

Locality: Pine Creek of Rock River, near Mt. Morris.

Collected: August, 1917.

Parthenita: redia.

This echinostome cercaria is named *Cercaria chisolenata* because of the crossing of the excretory tubules at the anterior end of each lateral system. The worm is 0.3 mm. long and one-third as wide. The tail is about 0.5 mm. long and 0.09 mm. wide at the proximal end. At the anterior end the collar prominence is provided with about forty small sharp spines consisting of a series in two alternating rows on the dorsal side of the worm and extending ventrad to a region just below the anterior end of the excretory system. Each spine measures about 20μ in length. The oral sucker is 44μ in diameter. A ventral sucker of equal diameter is situated a short distance behind the middle of the body. The tail is marked by longitudinal muscle fibers, which become less conspicuous distad.

The redia is a large parthenita, 1.5 mm. long and 0.45 mm. in section across the feet. These appendages occur one-third the distance from the posterior end of the worm. A collar prominence is found near the anterior end. Just behind it on the ventral side is the conspicuous birth pore. The wall of the redia is thick. At the anterior end there is a pharynx 10μ in diameter. Behind this there is a dwarf rhabdocoel gut, barely twice the length of the pharynx. Many cercariae develop within the redia at the same time. The larvae seem to develop in batches, so that the production tends to be rhythmical.

The excretory system is typically echinostome in character. The bladder receives the pair of lateral trunks through a common chamber. These trunks are most dilated just anterior to the acetabulum, where they are filled with granules. Just behind the pharynx they narrow down to the dimensions of capillaries. At the posterior margin of the oral sucker each capillary bends abruptly outward, then backward, crossing back on itself at the junction of trunk and capillary. Here it ends in a flame cell. One flame cell is also found in the outward bend and one at the reflexing of the capillary. Three flame cells such as these at the anterior end of the system are probably found in the majority of echinostome larvae. A single median tubule in the tail cares for excretory wastes in that organ.

The digestive tract is surrounded by a very small pharynx at its anterior end. The esophagus continues to the anterior margin of the acetabulum. Here the ceca arise and continue to the caudal extremity of the worm. There are two series of mucin glands, with many members to each series. They empty through common ducts into the oral atrium of the cercaria.

The genital organs are poorly developed. An ovarian cell mass is found just anterior to the bladder; another cell mass is found at the antero-lateral margin of the acetabulum. Fine lines of vitelline follicles are traceable anteriorly and posteriorly.

The animal is filled with small cystogenous glands, which arise late in the development of the larva through differentiation of indifferent parenchyma cells. The worm normally decaudates and encysts in the host tissue. The cyst wall is thick and firm, providing a safe abode for the worm until a transfer of hosts is effected.

Cercaria acanthostoma nov. spec. (Figs. 14-17)

Host: *Planorbis trivolvis* and *Physa gyrina*.

Locality: Urbana.

Collected: October 29, 1917.

Parthenita: redia.

This echinostome larva resembles the typical larvae of the group in having a very muscular body and an active tail, in the possession of a collar prominence with numerous spines, and in an abundant supply of cystogenous material. The body is 0.3 mm. long and 0.12 mm. wide, while the tail is 0.44 mm. long and 0.044 mm. wide at the proximal end. The oral sucker is 58μ in diameter and the ventral sucker is 65μ in diameter. A unique character of this larva is the group of six spines inserted in a single row in the roof of the oral sucker, with points directed forward. The larva is named *Cercaria acanthostoma* because of this oral spine group. The ordinary spines around the collar number from thirty-four to thirty-eight. They are sharp-

pointed and inserted in an irregular row. The tail is crenate along the margin. Although this feature is probably due to the muscular elements of the caudal organ, the wavy outline of the organ is a constant character.

The redia is comparatively small, 0.3 mm. long and 0.058 mm. in diameter. The feet are situated three-fifths the distance from the anterior end. A collar prominence is found a little way from the oral end. The pharynx is small and leads into an inconspicuous thick-walled rhabdocoel gut. The embryos develop at the posterior end of the parthenita. They press forward as they develop.

The excretory system in the body of *Cercaria acanthostoma* consists exclusively of a bladder and delicate tubules and capillaries. The bladder is biconvex with muscular walls. The pair of main tubules enters the bladder from the extreme lateral margins. Along the course of each tubule are thirteen flame cells, eight on the inner margin of the tubule and five external to the tubule (Fig. 14). At the extreme anterior end of the excretory system the tubule reflexes and then fuses to form a delta, at each angle of which there is one flame cell. Thus the total number of flame cells along the entire course of the tubule is sixteen. This system is much more delicate than the more common echinostome type with the large lateral trunks and capillary system. The excretory system in the tail is confined to a long sac-like reservoir extending the entire length of the organ without any definite wall or lining. Near the proximal end it frequently bulges out on each side to form a lateral reservoir. No flame cells were distinguishable in the tail.

The digestive tract has a prepharynx region, a large pharynx 32μ in diameter, an esophagus extending to the anterior margin of the acetabulum, and ceca extending as far posteriad as the excretory bladder. Mucin glands are present in a biserial arrangement.

The nervous system is well developed. The six main posterior trunks are visible in the toto mounts. The pair of posterior ventrals is unusually large.

The genital cell masses consist of a pyriform ovary and a vagina along the midline just in front of the acetabulum.

The animal is filled with numerous cystogenous glands, yet encystment is slow and infrequent. When it does occur the cyst membrane which is formed is thin and tough. Through it the spines and excretory system are visible.

Cercaria trivolvis Cort

Host: *Planorbis trivolvis*.

Locality: Normal School pond, DeKalb; drainage ditch, Urbana.

Collected: November, 1916; August, 1917.

Parthenita: redia.

This species was first described by Cort in 1914 and again in 1915. The writer has been able to examine the material from the same host in the same and in a different locality. Certain points of structure not described in the original accounts have been observed.

The specimens studied by the writer were somewhat smaller than those described by Cort. They averaged in body length 0.34 mm., while Cort's specimens had an average length of 0.38 mm. Their body width was 0.11 mm. as contrasted with Cort's width measurement of 0.12 mm. Likewise the tails of the writer's specimens were about 0.44 mm. as compared with 0.5 mm. in the individuals worked over by Cort. On the other hand the oral sucker and acetabulum of the DeKalb specimens measured 50μ and 58μ , respectively, as compared with 43μ and 49μ of Cort's material. This difference in size may be entirely dependent on the degree of maturity or on nourishment, while the size of the suckers may depend largely on the degree of expansion or contraction.

In addition to the characters described by Cort, the writer has found paired right and left clumps of salivary-mucin glands filling practically the entire body from pharynx to acetabulum. Each cluster is oval and consists of a very large number of minute cells with large dilated nuclei. These gland cells are similar to those described by the writer for *Cercaria reflexa* (1918, Fig. 134). They differ, however, in arrangement and distribution.

The crevices between the body wall and the salivary-mucin glands are packed with cystogenous glands, as is also the greater portion of the posterior portion of the body. The individual gland cell is polygonal and contains several elongate cyst granules. These granules may be compared to concentrated gelatin tablets, capable of enormous swelling by water inclusion, when the stimulus for cyst formation is at hand. Then by a rapid wriggling the tail is thrown off and a thick gelatinous cyst is secreted around the larva. Through this cyst none of the organs can be definitely made out. Only the large excretory granules are apparent through the cyst membrane.

STYLET LARVAE

Cercaria stilifera nov. spec. (Figs. 18, 19)

Host: *Physa gyrina*.

Locality: Pine Creek of Rock River, near Mt. Morris.

Collected: August, 1917.

Parthenita: sporocyst.

This cercaria is large for a stylet larva, 0.32 mm. in length, 0.17 mm. wide in the region of the acetabulum, and possesses a tail 0.23 mm. long and 0.07 mm. wide at the proximal end. There is a pair of caudal pockets at the junction of body and tail, with a small number

of sharp spines directed mesad. The oral sucker is large, about 85μ in diameter, while the acetabulum, slightly posterior to the middle of the body, is only 58μ in diameter. The quill inserted in the dorsal wall of the oral sucker is a simple structure, 32μ long, and reinforced only at the base.

The cercaria develops in a sporocyst of irregular contour, about three or four times as long as the transverse diameter of the parthenita. The shape of the sporocyst is largely dependent on the movement of the cercariae within the sac. The wall of the sporocyst is extremely thin and delicate and is ruptured with the slightest pressure. Immature cercariae will not live in half-saline solution.

The excretory system in the wall of *Cercaria stilifera* consists of a bladder slightly muscular, which changes in shape from oval to squarish in surface view, a single narrow reservoir directly anterior to the bladder, a pair of lateral cornua, and two main body tubules. One of these tubules is directed posteriad, the other runs anteriad just lateral to the acetabulum and at the anterior half of the worm forms two branches, the inner one of which ends in the region of the pharynx and the outer one of which ends over the oral sucker. All of these tubules have several tributaries, each one of which originates from a pair of capillaries. At the inner end of each capillary there is a flame cell. The excretory system in the tail consists of a long awl-shaped reservoir at the proximal end immediately behind the bladder and a single median tubule with some ten tributaries, each of which arises from the junction of the two capillaries.

The digestive tract is composed of a short, narrow prepharynx, a small pharynx 17μ in diameter, a long esophagus extending almost to the acetabulum, and a pair of short ceca which barely suggest the bifurcate nature of the system. Opening into the oral atrium at the extreme sides of the large sucker are the two groups of salivary-mucin glands. There are about twelve glands in each lateral group, each gland having an individual duct to the subatrial region. Here they all empty into an enlarged portion of the system filled with granules. A single duct connects this dilation with the oral atrium.

The genital organs are represented in *C. stilifera* by a knobbed mass of cells posterior to the acetabulum, a thick cord of cells on the left of the embryo just within the limits of the acetabulum, and a thick tubule just under the anterior half of the acetabulum. All of these are joined together in the order named. In addition conspicuous masses of vitelline masses are found at the sides of the body in club-shaped aggregates extending forward to the pharynx and posteriad to the bladder. The germ cells in the posterior part of the body lie ventrad to the excretory cornua; the cell mass more anteriad lies above the ventral sucker.

The nervous system is somewhat degenerate. In the region of the pharynx is a large mass of diffuse fibers, which constitute the ganglion mass. The nerve trunks are not easily distinguished.

Cystogenous glands are found scattered through the body. They are large and relatively few in number. Their nuclei are oval to spherical, and the granules in the cytoplasm are acidophilic, as contrasted to the basophilic cystogenous granules of other species. This gland structure is probably closely correlated with the slow decaudation of the animal and infrequent encystment, and suggests that the worm gains entrance to the next host either through active swimming or the next host eating the larva while it is yet within its primary host.

Cercaria isocotylea Cort (Figs. 20-24)

Host: *Planorbis trivolvis*.

Locality: drainage ditch, Urbana, and Normal School pond, DeKalb.

Collected: 1916-1917.

Parthenita: sporocyst.

This cercaria was originally described by Cort in 1914 and again in 1915. The writer's measurements for the species are far in excess of the original description, ranging from 0.2 to 0.32 mm. for body length and 0.1 to 0.12 mm. for body width, while the oral and ventral suckers both measure about 50 μ . The fact that Cort's specimens showed no cystogenous glands, together with their smaller size, suggests their immaturity. The tail in all of the specimens which the writer observed was small and not particularly active.

The stylet is described by Cort (1915: 54) as "sharp-pointed and has a thickening two-thirds of the distance from its base to its tip." The sharp, spinose portion of the stylet takes up the anterior third of the organ. The rest of the quill is set off by a nodular reinforcement on the dorsal side and is thickened dorsad toward the base (Figs. 22-24). From the base four tongue bars extend out anteriorad.

At the posterior end of the worm the caudal pockets include the proximal portion of the tail. Each member of this pair is provided with a small group of spines projecting inward. The absence of these spines in Cort's description is an additional point in favor of the view that his specimens were immature. The small body spines which extend over the anterior two-thirds of the animal decrease in size from the anterior tip caudad.

Figure 20 shows the distribution of the excretory tubules in the species. At the end of each capillary is a minute flame cell. Twenty-two flame cells have been counted on each side of the body. A single unbranched tube is found in the tail.

The digestive canal has been described by Cort as undeveloped save for oral sucker, short prepharynx, and pharynx. The writer has

found a large group of gland cells in this species directly behind the pharynx. Further study has shown these cells to open into two short ceca and a miniature esophagus. These organs are at times so far dorsad that they are not in the same focus as the pharynx. The salivary-mucin glands (stylet glands of Cort) are regarded by the writer as a part of the digestive system. Their presence in echinostome and schistosome larvae, where no stylet is present, and the chemical nature of their content, make the theory of their salivary nature altogether probable. The writer has counted nine of these gland cells in each lateral group.

The genital cell masses are found dorsal to the acetabulum. On the posterior margin are found three masses, a median oval organ, the ovary, and two lateral lobed organs, the testes. Two masses dorsal to the anterior margin of the acetabulum correspond to the vagina and the cirrus sac. Stretches of vitelline glands occupy the sides of the body from the region of the pharynx to the extreme posterior margin of the body.

The cystogenous glands are similar to those of *C. stilifera*, few and vesicular. The granules are small. Decaudation occurs seldom. Encystment has been found to take place only within the host tissue. The cyst membrane is probably secreted very slowly, in contrast to that in monostomes and some echinostomes. This cyst wall is thick and gelatinous.

The topography of the various organs in these stylet cercariae, *C. stilifera* and *C. isocotylea* Cort, suggest plagiiorchiine relationships. While the writer believes that the stylet *per se* is a very general character which may be found only in larval Plagiiorchiidae or may, on the other hand, be found in other related Distomata, the opening of the genital pore anterior to the acetabulum is a more specific feature of these forms to which attention is directed. The stylet larvae previously described by the writer (Faust, 1918) have been found to possess cirrus or vaginal cell masses which open into a genital atrium anterior to the acetabulum. Magath (1918) has recently shown that a stylet cercariae in *Planorbis trivolvis* at Fairport, Iowa, develops into a worm related to the Plagiiorchiidae but differing from the Plagiiorchiidae in having a lateral genital pore. For this he has proposed the new subfamily Lissorchiinae. Unfortunately he was unable to make out any of the genital complex in this cercariae except the ovarian cell mass, so that it can not be compared item for item with those described by the writer. However, among other things, they possess in common (1) a stylet set in the dorsal wall of the oral sucker, (2) paired mucin glands, and (3) sporocyst parthenitae. A striking difference between the excretory system of the cercaria of *Lissorchis fairporti* and the plagiiorchiine cercariae is the flexing back

of the main anterior tubule in the former species and the absence of such flexing in the latter larvae. On the whole the larvae of these two families show marked relationships.

SCHISTOSOME LARVAE

Cercaria gigas nov. spec. (Figs. 25-30)

Host: *Planorbis trivolvis*, *Physa gyrina*.

Locality: Normal School pond, DeKalb; Pine Creek of Rock River, Mt. Morris; drainage ditch, Urbana.

Collected: August-November, 1917.

Parthenita: sporocyst.

This larva is a giant among schistosome larvae. Its body length is 0.28 mm., its width 0.09 mm.; the unforked portion of the tail is 0.32 mm. long, and the tail furci, 0.18 mm. Iturbe and Gonzalez (1917) have stated the measurement of the cercaria of *Schistosoma mansoni* to be as follows: body length, 0.1-0.13 mm.; breadth, 0.04-0.05 mm.; unforked tail, 0.14-0.15 mm.; furci, 0.04-0.05 mm. The writer's measurements on some of Iturbe's material shows a slight excess in all of these measurements. *Cercaria douthitti* (Cort, 1915) has a body length of 0.19 mm., and an unforked tail length of 0.22 mm., while the furci measure 0.089 mm. *Cercaria tuberistoma* (Faust, 1918) has a body length of 0.2 mm., and a combined tail length of 0.32 mm. O'Roke's *C. echinocauda* has a body slightly larger and a tail nearly twice as long (1917).

Cercaria gigas is characterized by a pair of pigment eyes on the dorsal side, about two-fifths the body distance from the anterior end; by a small ventral sucker, 26μ in diameter; by a long unforked portion of the tail which is muscular to an extraordinary degree, and by fluted borders to the furcae. On account of a pronounced flexure at the juncture of body and tail, the animal is more often seen on the side than on the dorsal or ventral surface. In this attitude it has a characteristic irregular appearance more easily pictured than described (Figs. 28, 29). The oral sucker sticks out antieriad like a snout. It is covered with minute spines and is invertible. The acetabulum protrudes some distance ventrad. Behind it a group of gland cells bulges ventrad. These glands are easily made out in the living animal as an oval mass of yellowish-white in the midst of a grayish background.

Often the ventral surface is streaked with pigment, especially just behind the eye-spots. Melanoidin granules are frequently distributed over both dorsal and ventral surfaces.

The unforked portion of the tail is large and powerful. Many longitudinal muscle fibers run the entire length of the organ. These are reduced in size and number in the furcae, which are thin and paddle-like with their edges directed dorsoventrad.

The sporocysts are long, irregular sacs, most usually pointed posteriorly and muscular in the anteriormost portion. The walls of the sporocyst are moderately thick. The cercariae appear to develop in batches.

The excretory organs have been worked out in detail in *Cercaria gigas*. The bladder is small and oval. Two main tubes enter into it side by side at its anterior end. These reach forward to the region of the eye-spots. Along the course of each tube are ten flame cells. A main tube in the unforked portion of the tail receives a tubule from each furca. At the proximal end of the caudal organ a lateral tubule on each side flows into the main tube. At the head of each lateral tube is a flame cell. The tube splits and reunites just before it enters the bladder.

Cercaria gigas has no pharynx. Esophagus and ceca are also wanting. Very large ducts with thick walls empty into the sides of the oral sucker. These ducts are the openings of two paired groups of gland cells. The anterior of these groups consists of several gland cells centering around the acetabulum. The protoplasm of these cells is granular and the nuclei are small. The posterior group consists of many cells, small and chromophilic. All of these glands are salivary-mucin in character. Their large number is unique among Schistosomatid larvae.

The nerve tracts are well defined. Anteriorly there are three main pairs of trunks. Posteriorly the laterals are lacking and the dorsals soon fuse with the ventrals. The eye-spots have a direct connection with the anterior dorsal trunks.

Only one group of genital cells is found, the testes mass, just behind the acetabulum.

The cercaria does not encyst. It probably reaches its definitive host by direct method and bores its way through the tissues to the blood stream.

By gross inspection *C. gigas* is likely to be confused with *C. echinocauda*. This resemblance of the two species is pronounced, save for the longer tail stem in *C. echinocauda*. Possible confusion of these two forms warrants a discussion of their similarities and differences. The writer has been fortunate to secure material from O'Roke and has therefore been able to check up the items from the material itself. *C. echinocauda* is longer and wider; its tail length is disproportionately greater. The furcae of both species have about the same measurement. But while the furcal fins of *C. gigas* are closely fluted, those of *C. echinocauda* are flat and braced with radial thickenings so that they were mistaken by O'Roke for spines. The longitudinal muscles in the tail stem of both species are prominent, but they are coarser in *C. gigas*.

In *C. echinocauda* the furcae arise slightly lateral, with a stub of the tail stem extending slightly distad; in *C. gigas* the furcae arise from a common center in the midline. O'Roke (1917:171) mentions the flexure at the junction of body and tail of *C. echinocauda*. This flexure is much more pronounced in *C. gigas*, so that it is difficult to get a frontal mount.

C. echinocauda has no spines at the oral end of the body such as are found on *C. gigas*. The pigment eyes of *C. echinocauda* are cup-shaped, with the opening dorsolateral; the eye-spots of *C. gigas* are long, sac-shaped organs, with the long axis extending dorsoventrad. No pigmentation other than that of the eyes has been observed by the writer in *C. echinocauda*.

Internally the structural differences of the two species are pronounced. The oral pocket in *C. gigas* ends blindly; there is neither pharynx nor esophageal glands. In *C. echinocauda* there are a few attenuate esophageal glands at the base of the oral pocket. The mucin-gland ducts in both species are large and conspicuous; but while there are two structurally differentiated groups of mucin glands in *C. gigas*, with many glands in each group, there are a few large glands of only one kind in *C. echinocauda*. The latter are chromophobic. The testes cell mass in *C. gigas* is composed of several small entities immediately behind the acetabulum; in *C. echinocauda* relatively few units compose this germinal mass and the gland is a considerable distance behind the acetabulum. Moreover, other genital cell masses may be made out distinctly in the region of the acetabulum of the latter species. O'Roke has not made out the excretory tubules or flame cells in the body of *C. echinocauda* so no comparison of these organs in the two species can be made.

C. echinocauda is described as the offspring of a redia, whereas the evidence of studies on other cercariae of the furcocercous group preponderates in favor of the development of these cercariae within sporocysts. Such sporocysts are at times muscular at the anterior end, with a pouch-like structure which serves as a sucker, but in no case has a true pharynx or rhabdocoel gut been demonstrated. The material of *C. echinocauda* examined by the writer contains no parthenitae, but the general outlines of O'Roke's figures (1917, Figs. 37, 41, 47) suggest sporocysts rather than rediae. This question must be carefully checked before it is finally settled.

Cercaria minor nov. spec. (Figs. 31-33)

Host: *Physa gyrina*.

Locality: Normal School pond, DeKalb.

Collected: August, 1917.

Parthenita: sporocyst.

Cercaria minor is much smaller than *C. gigas*. The length of the oval body is 0.14 mm. and the width, 0.068 mm. The unforked tail measures 0.2 mm., which is the same length as the furcae. The tail has a transverse diameter of 40μ at the proximal end. The oral sucker opens ventrad. It is 23μ in diameter and considerably deeper. The ventral sucker is in the posterior half of the body. It is 26μ in diameter and has a small circlet of spines within its margin. A pair of non-pigmented eye-spots is found in the region posterior to the oral sucker. Large parenchyma cells are found in the unforked portion of the tail.

The sporocyst is large and irregular, measuring up to 2.1 mm. in length by 0.27 mm. in diameter. One end is slightly muscular and is used in burrowing.

The excretory system consists of an oval bladder, flattened antieriad, and a pair of main tubules which stretch antieriad to the region of the oral sucker. Each tubule gives off two biramous inner branches and a single biramous posterior twig. The tail has a single unbranched median tubule. An eyelet anastomosis occurs between the tail and the bladder.

The pharynx is represented by a few small glandular cells. Four pairs of salivary mucin glands empty into the oral sucker thru heavy ducts.

Cercaria minor has not been found to encyst. It probably reaches the definitive host as a cercaria and then metamorphoses into the adult schistosome.

In an attempt to harmonize the flame cells of furcocercariae, Cort (1918) has recognized three divisions of these larvae: (1) those characterized by absence of a pharynx, tail furcae less than half the length of the tail stem, eye-spots present; (2) human schistosome larvae; (3) those with pharynx present, tail furcae almost as long as main stem. According to this grouping, *C. gigas* falls into the first class, altho it possesses ten pairs of flame cells in the body and one pair in the tail, a larger number than is found in Cort's forms, *C. douthitti* and *C. elephantis*. While *C. minor* (Figs. 31, 32) bears some resemblance to *C. douglasi*, it is much more akin to *C. gracillima* in possessing non-pigmented eye-spots and pyriform glands in the region of the esophagus which definitely denote the transformation of the pharynx region from a muscular to a glandular organ. Moreover, *C. douglasi* is classed outside of the Schistosomatidae because it has a pharynx, while *C. gracillima* has been shown to possess a definite schistosome nervous system (Faust 1918: 54). With the broadening knowledge of schistosome larvae, it seems more reasonable to recognize a complete series of larval forms from those with a pharynx sphincter (*C. douglasi*, *C. emarginatae* and perhaps *C. vivax* Sonsino), thru those with a degenerate pharynx, with or without intestinal ceca (*C. gracillima*,

C. minor), thru those without any pharynx, but with well developed mucin glands (*C. gigas*, *C. tuberistoma*, *C. douthitti*), to the human schistosome cercariae. For example, *C. minor*, in lacking intestinal ceca, is more closely related to *C. douthitti* than *C. gracillima*. Yet the eye-spots in *C. minor* are not pigmented.

Until the genital cell masses of each of these larvae have been carefully studied it is useless to attempt the relationships within the groups.

Thru the courtesy of Professor Henry B. Ward, the writer has been enabled to examine specimens of *Planorbis quadrelupensis* Sowerly infected with schistosome larvae sent by Dr. Juan Iturbe of Caracas, Venezuela. One vial of this material (No. 17.198 Ward collection) with the accompanying label, "rediae in state of development," was found to contain no stages in the life cycle of the schistosome larva, but instead a unique tetracotyle, for which the name *Tetracotyle iturbei* is proposed.

Tetracotyle iturbei nov. spec.

T. iturbei is a pyriform fluke, 0.42 mm. in length, 0.33 mm. in width, and 0.3 mm. in thickness. The oral sucker has a diameter of 52μ , the primitive genital pore, 42μ , and the acetabulum, 95μ . Suctorial grooves, muscular in part, are located at the sides of the ceca. The worm is unarmed and is enclosed in a thin mucoid cyst. Prepharynx is lacking; the pharynx measures 16μ in trans-section; the digestive tract forks immediately behind the pharynx. The ceca extend thru the anterior half of the body. The ovary, measuring 25μ in diameter, lies midway between the acetabulum and the posterior genital pore. Vitellaria are massed into two compact chorda, which reach cephalad as far as the primitive genital pore. The ootype, dorsal to the ovary, leads posteriad thru a short duct into a small genital pouch. Large pyriform testes, 50μ in long diameter, lie lateral and somewhat anterior to the ovary. Separate efferent ducts lead into the posterior genital atrium. Anterior to the ovary is a vagina, connected by means of a coiled tube with the anterior ventral sucker, the expanded primitive genital pore. This species yields data in support of the view that the posterior, and usually degenerate, ventral sucker of holostomes is the acetabulum of distome species.

T. iturbei is figured (Iturbe and Gonzalez, 1917; pl. 1, figs. 1-9) as the redia of *Schistosoma mansoni*. Proof that Iturbe's "redia" is a distinct species of fluke confirms the belief that rediae are not found among Schistosomatidae.

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EXPLANATION OF PLATES

PLATE I

Cercaria robusta—Fig. 1.—Dorsal view, showing pigmentation, excretory system and longitudinal muscles of the tail. $\times 170$. Fig. 2.—Dorsal view, showing germ glands in body and parenchyma cells in tail. $\times 170$. Fig. 3.—Redia. $\times 34$. Figs. 4, 5.—Stages in encystment. $\times 75$.

Cercaria aurita—Fig. 6.—Dorsal view, showing pigmentation and excretory system. $\times 105$. Fig. 7.—Dorsal view, showing digestive and genital organs. $\times 105$. Fig. 8.—Redia. $\times 54$.

Cercaria chisolenata—Fig. 9.—Dorsal view, with cystogenous glands on right of diagram. $\times 105$. Fig. 10.—Diagram of genital cell masses. $\times 105$. Fig. 11.—Dorsal view of collar spines. $\times 170$. Fig. 12.—Detail of anterior tip of excretory system with three flame cells. $\times 370$. Fig. 13.—Redia. $\times 54$.

PLATE II

Cercaria acanthostoma—Fig. 14.—Ventral view, showing excretory, digestive, and genital organs. $\times 170$. Fig. 15.—Pattern of collar spines, dorsal view. $\times 333$. Fig. 16.—Detail of anterior tip of excretory system. $\times 500$. Fig. 17.—Redia. $\times 54$.

Cercaria stilifera—Fig. 18.—Ventral view, showing excretory, digestive and genital organs. $\times 170$. Fig. 19.—Stylet. $\times 370$.

Cercaria isocotylea—Fig. 20.—Ventral view, showing excretory, digestive and genital organs. $\times 170$. Fig. 21.—Lateral view, illustrating description of spines on surface of body. $\times 105$. Figs. 22-24.—Dorsal, lateral and ventral views of stylet. $\times 370$.

Cercaria gigas—Fig. 25.—Dorsal view, showing eye-spots and salivary-mucin glands. $\times 170$. Fig. 26.—Excretory system. Fig. 27.—Sporocyst. $\times 54$. Figs. 27, 28.—Characteristic lateral views. $\times 54$. Fig. 30.—Immature cercaria. $\times 54$.

Cercaria minor—Fig. 31.—Ventral view, showing salivary-mucin glands in body and parenchyma cells in tail. $\times 170$. Fig. 32.—Excretory system. $\times 333$. Fig. 33.—Sporocyst. $\times 34$.

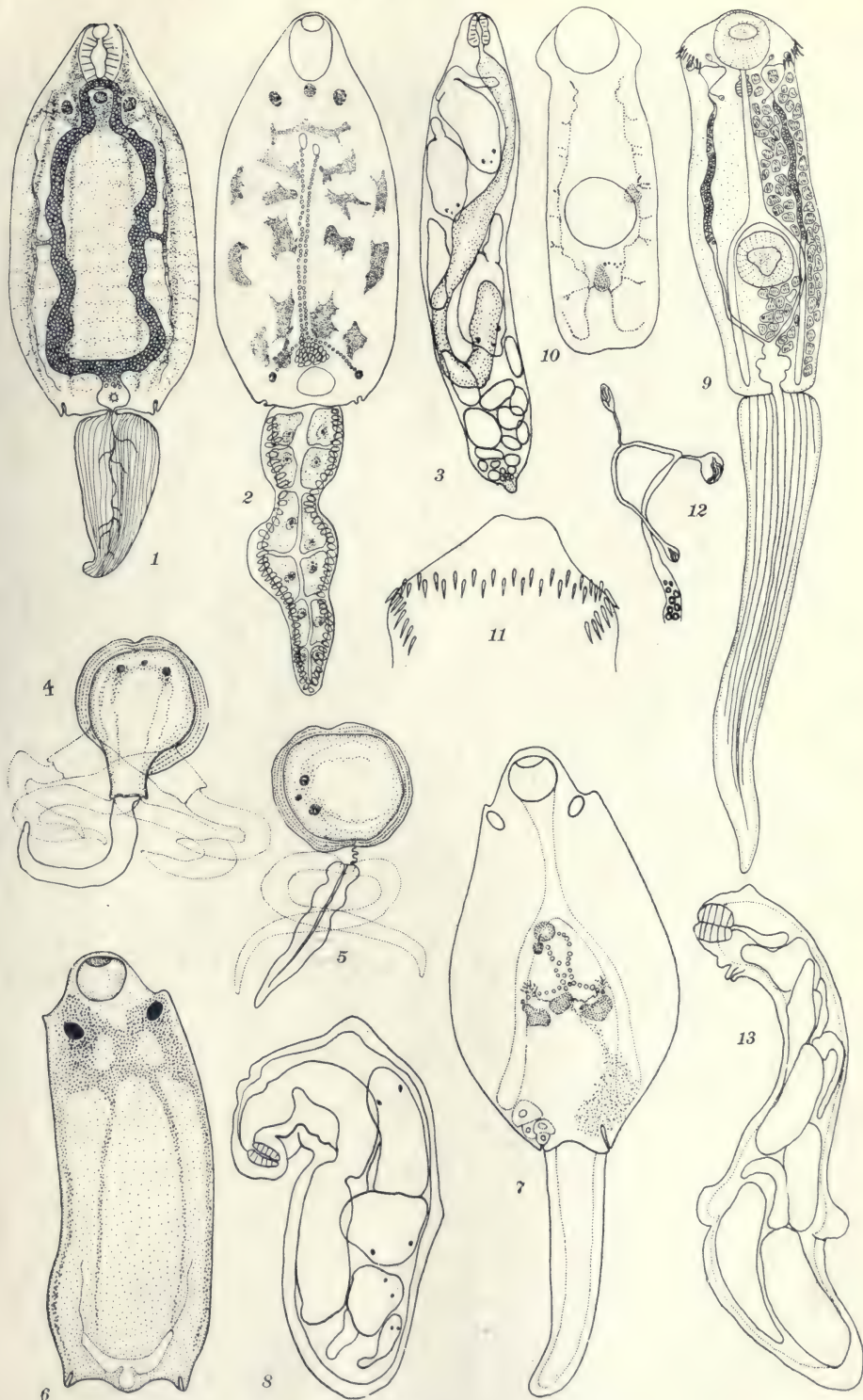
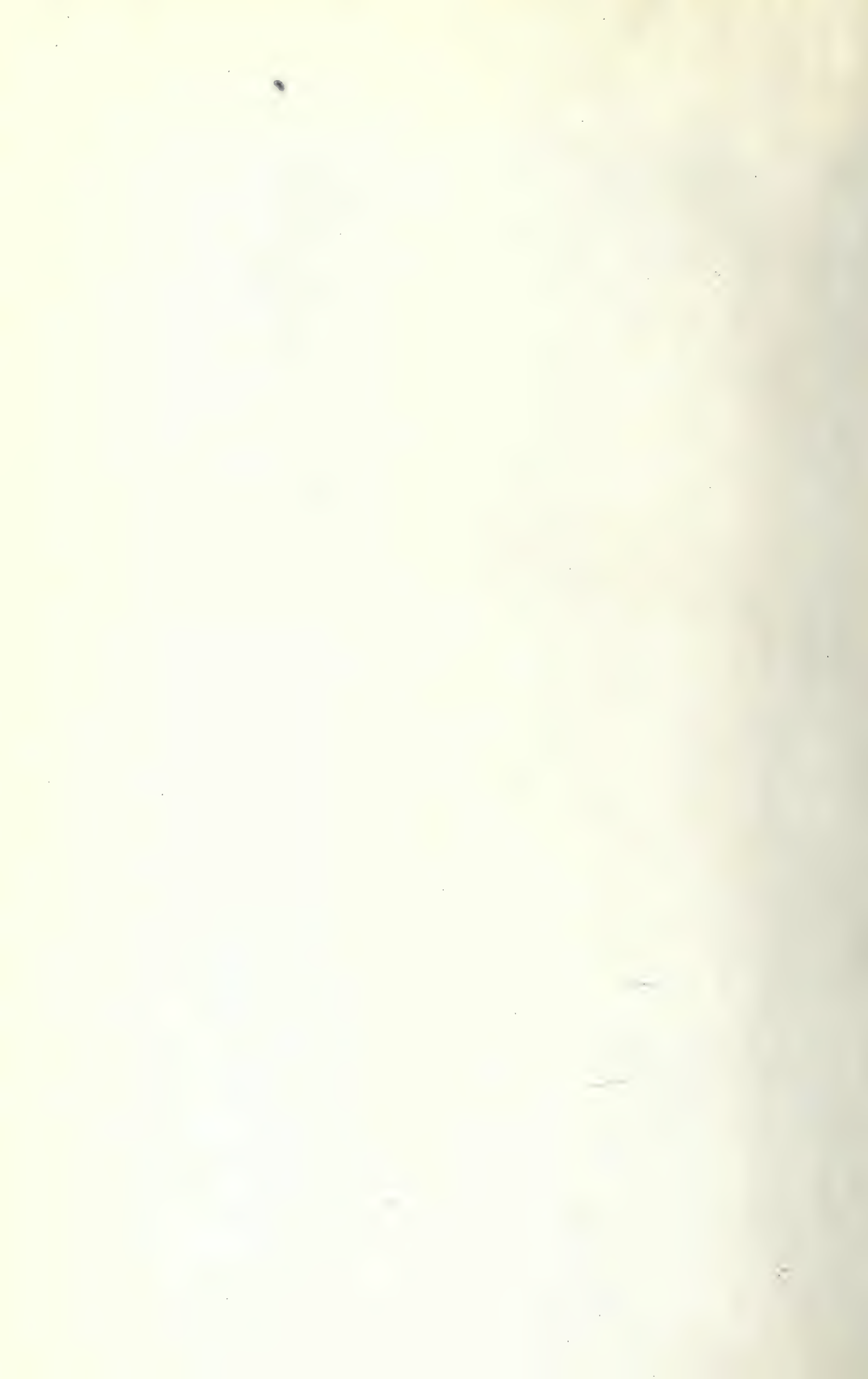


PLATE I



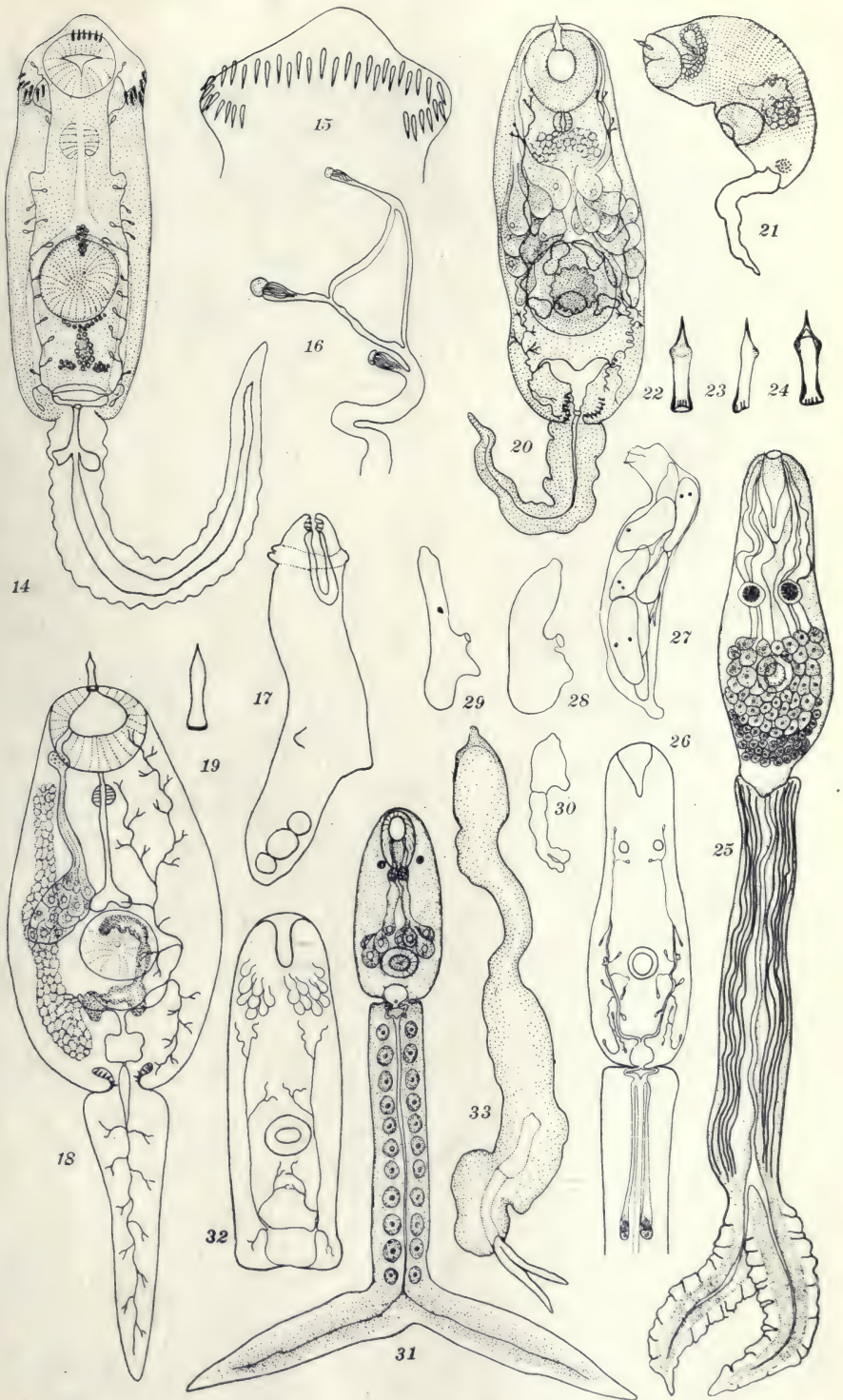
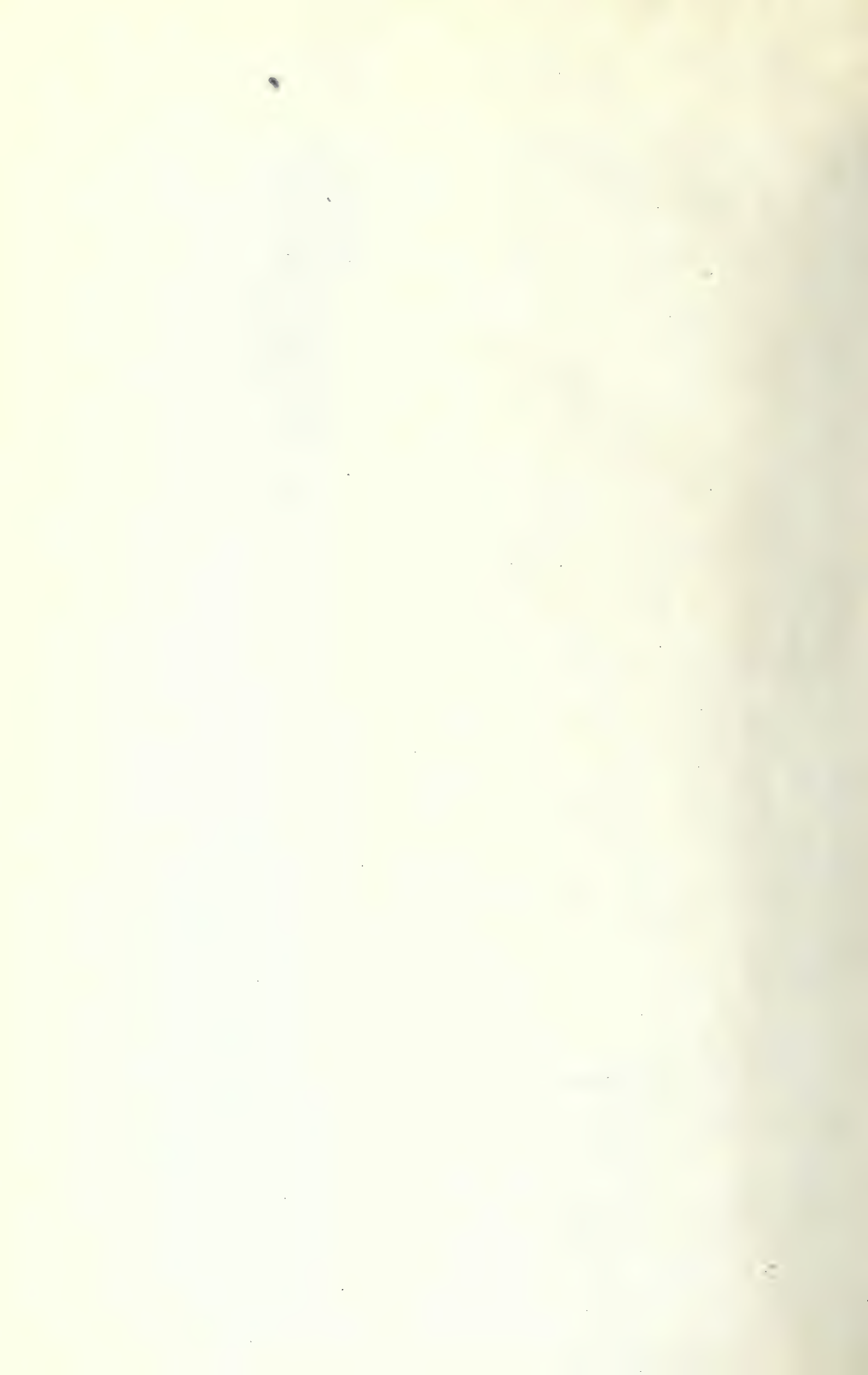


PLATE II



STUDIES ON THE SCREW WORM FLY, *CHRY SOMYIA* *MACELLARIA* FABRICIUS IN PANAMA *

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Throughout the Canal Zone and the Republic of Panama the screw worm fly may be found in great abundance and owing to its dangerous habit of depositing its eggs in living as well as on dead tissues of man and animals, is of considerable importance economically. The number of eggs deposited in one batch by each individual fly seems to vary considerably, but when all circumstances are favorable averages 190. Of a number of batches counted by the writer the minimum deposited at one time was 48 and the maximum 287.

When ovipositing in inanimate animal substances the females seem to evince a desire to lay their egg masses all together in a heap. If a number of females are confined in a jar containing a piece of meat and one deposits her eggs, either on or near the meat, the others then oviposit either next to or on top of the mass of eggs deposited by the first female.

The eggs of a single female are laid in an irregular mass or pile, usually being placed so they overlap or rest partly on top of each other after the manner of shingles. The time required for the eggs to hatch seems to be subject to some variation. The shortest time observed was 11 hours, the longest 23 and the average about 14 hours.

Surrounding conditions seems to exert but slight influence on this incubation period as moisture and temperature are, in a certain sense, always the same in Panama. The surrounding material whether in dead carcasses, decaying vegetable tissue, or live animal tissue, is in nearly all cases of a moist nature, at least sufficiently so to keep the eggs moist. Changes in the temperature of Panama are so slight that one may disregard their influence on the incubation period of eggs deposited on inanimate material. When eggs are deposited in animate objects the body heat may tend to shorten the incubation period considerably but there has been no opportunity to verify this opinion as no tests have been made with living animals. The variations seem to be primarily due to scarcity or abundance of suitable material in which to deposit the eggs and the consequent differences in the age of the embryos at the time the eggs are deposited.

* From a paper read before the Medical Association of the Isthmian Canal Zone July 20, 1917, to appear in full in an early number of the Proceedings of that Association; its distribution in that form will be limited and it contains much of interest to the parasitologist. W. A. R.

The growth of the larvae is very rapid, approximately 2 mm. a day until they become mature on the fifth or sixth day. There is then a period of about 24 hours during which the larvae feed but little and after which they leave the material on which they have been feeding to seek a suitable place to pass the pupal period. Under ordinary conditions this occupies a period of from four to five days but may be shortened to three days or extended to ten. Among the emerging adults there is little disparity between the sexes. In one lot of 450 flies, 42% were females and 58% were males. Sex did not exert any appreciable influence on the length of the pupal period.

The adult flies exist on fluids or semi-fluids in material that can be reduced to a semi-liquid food. This is generally found in garbage cans, refuse heaps, or other places of like nature near habitations, and in decaying animal and plant life or other odoriferous substances in the woods or jungle away from habitations. In the Canal Zone specimens have been captured while feeding on the syrup-like fluid in the decaying flower bracts of the *Heliconia* plants.

The females appear to prefer the late afternoon or evening to deposit their eggs. This raises the question whether or not they remain active after nightfall and fly about in search of places to oviposit. Most varieties of flies became inactive at nightfall, but I have known this species to deposit its eggs during the night while in captivity, and when gravid females are captured and placed in breeding jars, they will oviposit much more readily if the jar is covered with a dark cloth than if it is left uncovered.

In Panama this fly seems to be entirely impartial in its choice of environments. It shows a preference in its breeding places in so far as the material is concerned, but if suitable places are found in town and villages, the fly will be found there as well as in the jungle. In towns they may be found flying with an angry hum about decaying material left exposed, or around a corral containing cattle. The females are very active and always on the alert, searching for either food or a place to deposit their eggs. In the jungle they are quickly attracted by any animal, probably by its odor, but perhaps also by its motion. While passing along a trail they sometimes follow and fly around one's head with a vigorous buzzing noise, undoubtedly in search of a place to deposit their eggs.

ATTACKS ON MAN

It is safe to say that throughout the Isthmus of Panama this fly through the agency of its larvae causes a greater amount of damage and suffering to cattle, horses and other animals, than does any other dipterous pest found there, and if one excepts the disease-bearing mosquitoes the same also applies to man as well as animals.

Eggs are deposited in the human nasal and aural cavities and in other natural openings of the body as well as in every exposed wound, ulcer, and bleeding surface. This infestation usually occurs while the victim is sleeping in some exposed place. The nasal cavity is a favorite place of attack and also the most dangerous one for the victim. In these cases the person attacked generally has recently had an attack of nose-bleed with a clot of blood remaining in the nose, or has a nasal catarrh with an offensive discharge, or a foul-smelling breath.

Sleeping in the woods or jungle, and occasionally even in unscreened houses is fraught with danger. The odor of an offensive discharge or the scent of fresh blood is very perceptible to the strong sense of smell of this fly, and if any are in the vicinity they are soon attracted to the sleeper and proceed to deposit their eggs in his nostrils. As soon as the eggs hatch and the young screw worms emerge, they begin to migrate farther into the nostrils. They tear into the mucous membranes lining the nasal cavity, feeding on the blood and serum oozing from the small wounds caused by the chitinous head hooks. They soon penetrate into the sinuses of the nasal fossae, darker and moister regions. As the young maggots begin to feed they grow rapidly as they are in ideal surroundings for growth, namely plenty of liquid food, warm temperature, and a constant supply of fresh air. Under these conditions they are very active, soon devouring the membranes lining the nasal fossae and burrowing into the surrounding muscles and cartilages. The unfortunate victim suffers at first intense irritation which soon changes to very severe pain so that he undergoes considerable suffering.

In cases of nasal myiasis one often finds a history of a nose bleed at the beginning of the disease. This leads to the conjecture that the hemorrhage precedes the infestation and that the smell of blood attracts the fly which deposits her eggs at the first opportunity easily given if the individual lies down to sleep. In the opinion of the writer this is undoubtedly the manner in which infestation takes place in a great many cases, but it is very difficult to determine this point positively. Being attracted by an odoriferous catarrhal condition the flies may also deposit their eggs in the nostrils of a person having such a condition while he is asleep. The newly hatched larvae on burrowing into the membranes may tear open small veins and capillaries and cause hemorrhage, but in such a case the laceration of the blood vessels would be so gradual that the nose bleed would be but an oozing of blood mixed with serous fluid, and unlike an ordinary case of epistaxis.

Physicians coming in contact with nasal myiasis for the first time may often be at a loss for a diagnosis of the condition, and especially in a case where the worms are in the upper cavities or have burrowed so deeply into the tissues that they are not readily discernible. This

may not be until the larvae begin either to emerge from the patient's nose or to enter the mouth through the pharynx and to be expectorated. In tropical America a bloody, foul smelling, offensive discharge from the nostrils should arouse a suspicion that screw worms might be present.

A number of cases of human myiasis caused by *C. macellaria* have been reported from different parts of the United States, and Central and South America, and in but a few instances does the patient recall being attacked by flies. In a few cases it has been claimed that a fly had dashed into the nose while the victim was awake and moving about, but he was unaware that any eggs had been deposited. It is more than likely that in such cases a fly dashing at the nostrils was simply a coincidence and merely recalled on account of the worms being present in the nose and the patient being at a loss to account for the presence. The chances are a thousand to one that the fly he recalled, or imagined as trying to dash into the nose was innocent of depositing the eggs, which were probably those of another fly, deposited without knowledge of the person attacked. An imaginative patient could readily recall at any time the annoyance caused by a fly buzzing about his head.

A mass of 100 eggs of this fly measures about 4 mm. in diameter, or about the size of a small pea. Such being the case it seems highly improbable to any one who has made many observations on this fly and who is familiar with their egg laying habits and the size of the egg mass, that any normal person and especially any one having the nasal passages clear and being in the habit of breathing through the nostrils, could have a mass of eggs in the nostrils until they hatch without being aware of the presence of a foreign substance, except under certain conditions.

It is the opinion of the writer that infestation takes place in the nose under one of the following conditions: 1. A person goes to sleep in the late afternoon or early evening, and sleeps all night; the eggs are deposited in the early part of the evening and have a chance to hatch so that the young larvae ascend into the nostrils before the sleeper awakes in the morning. 2. During the day time a person who is intoxicated may lie down out of doors and remain in a drunken stupor for a number of hours, giving the larvae opportunity to hatch either while he is still in the stupor, or while the after effects of the intoxication on his head prevent the discovery of the eggs in his nose until after they have hatched. 3. An individual that has just had an attack of nose bleed and had a blood clot still remaining in the nares might very readily have a mass of eggs in the clot without being aware of their presence until after they had hatched. 4. A person afflicted with leprosy or any other disease causing anesthesia of the nasal

mucous membranes could very easily have eggs deposited in the nose while asleep and not know of their presence until they had hatched and the larvae had burrowed into sound tissue and caused pain. In cases where several hundred screw worms are found in the nose it is very evident that the victim was asleep, and the fly was unmolested for some time while depositing the eggs.

The writer has been informed that in the interior and remoter parts of Panama, it is not uncommon to find among the natives or Indians cases of nasal myiasis which terminate fatally. A large percentage of these cases have become infested after the individual had visited some town or village, drank heavily, and started for his home in a state of intoxication. The patient has a number of miles to travel on foot, horseback, or in a cayuca. The liquor making him drowsy he lay down in some shady place to sleep, and having a bloody surface in the nose or suffering from a nasal discharge he became a good subject for infestation.

Screw worm infestation is frequently in the ears, although records seem to show aural myiasis is somewhat less frequent than nasal myiasis. Babies and other persons that are somewhat defenseless, and whose ears are discharging, or are neglected and allowed to become dirty and emit an odor, even though sometimes very slight, are the ones that usually become infested. In these cases if the worms are not detected at an early stage they penetrate into the middle and inner ear, causing considerable suffering and sometimes death.

Infestation of the genitalia takes place occasionally. This usually happens among naked children, though it sometimes occurs in old people, who through senility or other causes, do not keep themselves properly protected with clothing at all times. All open wounds, sores, ulcers, and abrasions of the skin in man if left unprotected by dressings or clothing also prove to be good places for the female of this fly to deposit her eggs. The smell of blood in a fresh wound as well as any suppurating sore serves as an attraction. As a matter of fact, this species seems to be greatly attracted by any animal odor and will deposit its eggs in most any place on either man or animal whenever a favorable opportunity presents itself.

A normal umbilicus is sometimes selected as a place for oviposition. A case of umbilical myiasis was encountered at the Ancon dispensary on January 10, 1916, when a white Spanish laborer came to complain of soreness in the umbilicus. Dr. S. L. Van Valsah, formerly of this hospital, examined the man and found in the umbilicus three fly larvae which he extracted. He was kind enough to send both the larvae and patient to the laboratory for me to examine. The patient was a clean, well kept man of middle age, and although the umbilicus was quite deep and nearly closed there was no apparent odor emanating. It had

a very red and irritated appearance and the man complained it was very sore and tender to touch, but the skin was not pierced and there was no blood showing. He stated that for several weeks he had been one of a gang of men engaged in cutting grass and bushes, and clearing land at Corozal. He had finished with this work on December 31, 1915. The following day, January 1, 1916, he felt something "molesting" him, as he termed it, in the umbilicus which gradually increased until the soreness caused him to visit the dispensary on January 10.

This man claimed that he lived in a screened house at Balboa; that he always wore a shirt while working; that he had not lain down to sleep during the day time; and also that he had never observed any fly alighting on him anywhere near the umbilicus, so that it remains a mystery as to when he became infested. Very probably he had either laid down and fallen asleep during the day time and did not care to acknowledge it, or a fly had entered his quarters either through a hole in the screening, or through a door which had been left open. Many of the Spanish laborers while at work wear but a light undershirt with no overshirt. This might have been torn or loose enough to allow a fly to reach the umbilicus while he was asleep. At any rate, infestation had taken place without his knowledge and undoubtedly while he was asleep.

Two of these larvae were rather small, but the third one was large and mature. They were bred out and proved to be *C. macellaria*. Evidently these larvae had been in the umbilicus for nine days, living on the exfoliated skin debris, perspiration, and such serum as possibly exuded from the tiny scratches made by the head hooks of the larvae. In this case there was no blood, or suppurating or raw surface to attract the fly. The only attraction was probably the odor of living flesh, or some odor that might have been thrown off from the umbilicus when the man while working became warm and perspiring. It is the opinion of the writer that an annual survey of all the towns and settlements throughout the Republic of Panama would disclose a surprising number of deaths due to screw worm infestation, either as a principal or contributory cause.

ATTACKS ON ANIMALS

Besides man nearly all kinds of domestic animals, and some of the wild animals are subject to attacks from this fly. It has been recently discovered that this fly will deposit its eggs in the nostrils of perfectly healthy dogs. Dr. H. C. Clark, pathologist at this laboratory, informed me that he had examined a dog a few months previous that had been infested in this manner. This dog was a male deer hound and one of a pack owned by a hunting club at Ancon. The pack had

been taken to the Province of Chiriqui on a hunting trip and the dog became infested in the nostrils while there. After doing considerable damage by eating through the tissues into the mouth, the maggots were all removed and the dog saved.

When brought back to Ancon, a few weeks later, the dog was brought to the laboratory and examined by Dr. Clark. The worms had caused a loss of bones in the upper right half of the mouth and nose, loss of teeth in the upper right jaw, and partial or complete loss of smell. The health was recovered in other respects. The dog was of no further use in hunting, as the destruction of the sense of smell incapacitated him for following a trail. The screw worm may be classed as an important enemy of dogs on the Isthmus, not alone owing to the actual amount of tissue it destroys, but also on account of giving a favorable field for micrococcus infection which often follows attacks of myiasis and causes death.

Cats lick and clean all wounds that they can reach so persistently that larvae seldom have any chance to live in a lesion, but one cat has been observed that had two full grown screw worms in an open wound at the base of the skull just back of the ears. In this location it was impossible for the cat either to lick them with her tongue, or to remove them by rubbing with her paws. These worms have also been found in neglected spur wounds on fighting cocks. It is not known whether this is a common occurrence or not but one case was noted personally in the city of Panama.

Three tame deer that were kept in a yard at the laboratory a short time ago all became infested from very insignificant wounds. One male had worms in a wound near the base of the horns which in the beginning was nothing but a small place where the skin had become chafed and broken by a rope tied about the horns. The second one became infested in a wound on the hind leg near the knee which was originally a small nail wound. The outer opening in the skin remained very small, but the cavity beneath was quite deep, reached to the bone and followed along side of it for a short distance down the leg. Fifty-two larvae were removed from this wound. The third deer acquired maggots in a small wound on the nose caused by running against the fence.

EMERGENCE OF LARVAE WHEN BURIED IN THE GROUND

It is manifest that when a horse, cow, steer, or other animal dies and is left for a few hours before being disposed of, many eggs of this fly are deposited in the mouth, nose and other cavities. If burial is the method of disposal and is delayed beyond the time required for the eggs to hatch, the cavities will contain many young larvae that

will be buried with the cadaver. Even if the eggs are not hatched before burial their hatching is delayed but little, and the young larvae emerge as readily under the ground as on the surface.

After a cadaver is interred decomposition takes place somewhat more slowly than when it is exposed to the open air. This delay combined with the fact that the ground absorbs a large amount of the gases thrown off enables the larvae to live in the cavities, and to find a sufficient supply of oxygen to insure their reaching maturity.

In order to investigate the depth at which the larvae may be buried and still live, develop and emerge from the surface as adults, seventy-five larvae about half matured were placed in a wide-mouth bottle containing a piece of fresh meat. The bottle was left unstoppered and placed at the bottom of a box about five inches square and three feet deep. A mixture of clay and sand was placed on top of the bottle to the depth of two and a half feet. This earth was tamped to make it as compact as undisturbed ground would be. A wire screen cover was then fastened securely over the open end of the box. The box with the closed end containing the larvae downward was set in the ground, the earth was replaced around the box and packed tightly to equalize inside temperature, moisture, etc., a few inches of the box and the screen cover projected above the surface of the ground, and was inspected each day thereafter for adult flies.

Ten days following the burial of the larvae the first adult flies were found in the cage. They continued to emerge up to the fourteenth day, and a total of forty-two emerged during the four days. This shows that 56 per cent. of the buried larvae lived to complete their metamorphosis and reach the open air as adults. Eighty-four per cent. of the number that emerged were females.

When the carcass of a dead animal is buried, it is seldom that it is covered with more than two and a half feet of earth; while 56 per cent. of the larvae buried with the carcass emerge at this depth, the percentage that emerges when the carcass is buried at lesser depth must be proportionately greater.

TRANSMISSION OF DISEASE BY THE FLY

Up to the present no positive proof has been found that will serve to incriminate the species as a disease-transmitting agent. However, work along this line is advisable, especially as regards the transmission of anthrax among cattle. When an animal dies of anthrax a thin blood stained fluid is usually sprayed from the mouth and nostrils. If in a pasture, the animal is generally stretched out on the ground, and when dying the ground in front of the head is sprayed with this liquid and the face around the nose and mouth covered with it. Cattle

dying of anthrax seem to bloat very quickly and in some cases the odor of decomposition is noticeable shortly after death even while the bloody spray is still wet on the muzzle of the animal, or on the grass in front of it.

In localities where the screw worm flies are numerous they are attracted to an animal succumbing to anthrax very soon after death has occurred. In such cases they are found either feeding on the discharged fluids on the ground or face of the animal, or are busy depositing their eggs about the mouth, nose, anus, vulva or other place where there is sufficient discharge to produce a moist surface.

In Panama the buzzards locate the body of an animal soon after death and after a few of these scavengers start feeding on a carcass it presents even better opportunities for the flies.

In April, 1916, the writer viewed the carcasses of three steers that had died of anthrax in a pasture near Colon. They had been dead only a few hours but had begun to bloat and a strong odor of decomposition was emanating from them. A veritable swarm of screw worm flies was feeding and ovipositing on each carcass, although they were nearly a mile apart. When these flies either feed or oviposit on a carcass the feet and proboscis must necessarily become contaminated and in my opinion they are capable of infecting any animal that they may visit shortly after; that is, providing the animal happens to have a fresh brand mark that is unhealed, cuts from barbed wire, horn wounds, or any skin abrasions.

It is claimed that the anthrax bacilli will survive in the ground for several years and that even if all cattle are removed from a pasture that has become badly infected and it is left empty for several years, the infection may be still in the ground. If cattle are pastured over this ground even after five or six years a fresh epidemic is liable to break out. If the *Bacillus anthracis* is able to survive the rays of the tropical sun in the soil of Panama for several years it is certainly able to live on the feet and proboscis of the screw worm fly for a few hours or even days. It is plausible to believe that this fly may be one of the principal carrying agents in tropical and subtropical countries and it is hoped that observations may be carried out to test this theory of transmission.

BREEDING OUT LARVAE FROM CASES OF MYIASIS

Although the screw worm fly is the principal culprit it is known that other flies have the habit of depositing their eggs or living larvae in open wounds or on exposed parts of the body. It is to be deplored that in a great many cases of human myiasis the larvae are never identified or bred out to ascertain whether they are really *C. macellaria* or some other variety of fly. It is therefore suggested to physicians

called to attend a case of myiasis, that before washing the wound with any solution they remove specimens of the larvae from the lesion by means of forceps, with as little injury as possible, and place them in a glass jar containing damp sand and a piece of meat or decayed vegetable. The jar should be covered with a piece of muslin drawn tightly over the top and fastened with string or a broad elastic band. Beyond noting if more food is needed nothing else is necessary except to watch for the emergence of the adult flies. When they appear a few drops of chloroform may be poured on the muslin top which should then be covered with a piece of pasteboard or other flat object to retain the chloroform vapor in the jar. After exposure to this for a few minutes the flies may be removed and examined.

If this procedure could be carried out in all cases of cutaneous myiasis it might incriminate other flies on the Canal Zone, hitherto unsuspected, of having the same propensity for breeding in living flesh. *C. macellaria* is easily the principal offender in cases of nasal myiasis.

PREVENTIVE AND CONTROL MEASURES

Tests which are detailed in the complete paper were made with different drugs and chemicals to determine their respective lethal effects on the screw worm. From twenty to twenty-five worms, all of which were approximately mature, were used in testing each substance. The agents giving the most satisfactory lethal action were fat solvents which readily penetrated and dissolved the fatty tissue of the larvae.

Owing both to the great diversity of the breeding habits of this fly, and to the peculiar conditions existing in Panama, but little can be said in regard to its control. In caring for animals with infested wounds all open lesions may be sprayed with chloroform or carbon tetrachlorid to remove the maggots. Both of these have proved efficacious.

Carbon tetrachlorid is as fatal to the maggots as chloroform if not more so, it is equal in penetrating power, does not evaporate any more readily, produces no more irritation to the tissues, does not retard healing any longer, and is much cheaper. Carbon tetrachlorid does not attract the flies by its odor as the carbon bisulphide is said to do, and it also lacks the inflammability of the latter.

In deep punctured wounds it may be best to spray with glycerine first to cause the maggots to become active and approach the outer opening of the wound. They may then be sprayed with carbon tetrachlorid to destroy them. If one of the lethal fat solvents is injected in a deep wound in an undiluted state the larvae are apt to be killed before they are able to leave the wound, and remain as a foreign body causing suppuration. After a wound on an animal has been

cleaned of all screw worms it should be dressed with one of the repellent agents to keep the flies from depositing more eggs in the wound. Pine tar is a good repellent.

An excellent protective dressing may also be made by mixing equal parts of beeswax, fish oil, and carbon tetrachlorid, working in enough vaselin to give it the proper consistency. If all animal wounds, both those which are fresh and those from which the screw worms have been removed, are painted with this mixture it will prevent the flies from depositing their eggs and save the cattle from damage as well as reduce the number of the flies.

All fresh meat should be screened to prevent it from becoming blown and if necessary the screen may be reinforced with cheese cloth.

Lastly, all parties camping in the jungle should sleep under mosquito netting. This especially is necessary for persons having a nose bleed or catarrhal condition. And it should be remembered that it is of more vital importance to use the mosquito netting while taking siestas during the day than at night, as far as protection against this fly is concerned. A number of people who would use a netting at night as a protection against mosquitoes would scorn to sleep under it during the day time, but it must be remembered that the screw worm fly apparently deposits her eggs during the daylight hours, or at least before it gets very dark.

METHODS OF ASEQUAL AND PARTHENOGENETIC REPRODUCTION IN CESTODES

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In the following paper we propose summarizing the various facts, known at present, regarding the different methods by which the Cestoda reproduce themselves, asexually and parthenogenetically. The formation of secondary bladders in the parent cysticeroid, like what occurs in some of the common forms such as *Coenurus*, *Echinococcus* and *Polycercus*, is, of course, quite well known, but though some of the more uncommon types have been described carefully, they have never received that attention which they merit. Recently, two special types of budding in larval cestodes have been described, one by Ijima (1905) and the other by Beddard (1912). These instances, together with a new case of parthenogenetic reproduction, in what we believe to be a new and adult worm recorded by us (1917), suggested the desirability of reviewing those reproductive methods (other than sexual) known to occur in the Cestoda both in the larval and adult stages. We propose to discuss each case separately, beginning with larval forms in which such reproduction takes place.

TYPE A.—WITH INTERNAL BUDDING BY PROLIFERATION

1. *Monocercus* Villot.—In this genus a very primitive condition in the pro-scolex, or blastogen, gives rise to a single caudal bladder by a typical method of endogenous budding.

This is to be seen clearly in the species *Monocercus* (*Cysticercus*) *arionis* (Siebold). Villot describes an original connection between the posterior part of the caudal vesicle of the cysticeroid and the cyst, in the form of "une sorte d'ombilic ou de depression infundibuliforme." In *M. didymogastri*s Hill no original connection can be seen in the fully formed cysticeroid (Fig. 1). This certainly appears to be an advance on the condition in *M. arionis* described by Villot and leads to the condition in *Polycercus* which will now be considered.

2. *Polycercus* Villot.—The generic name *Polycercus* was proposed by Villot in 1883 for a cystic worm described in 1868 by Metchnikov. The species *P. niloticus* was so named by Willey (1907) because the

adult tapeworm stage of Metchnikov's larva is now known to be *Taenia nilotica* Krabbe, 1869, which is parasitic in *Cursorius europeus*. In its mature condition this species consists of a thin skinned bladder which contains a varying number (up to 13) of small cysticercoids of about 0.5 mm. in diameter. Although the latter lie quite free in the interior of the cyst and possess like the ordinary cysticercoids the distinctive caudal bladder, they are of very unusual origin, inasmuch as instead of developing directly from the six-hooked embryos, they arise by proliferation of the internal wall of the surrounding bladder (Fig. 2). The bladder is thus the brood capsule of the enclosed cysticercoids and corresponds in some respects to the brood capsule of the *Echinococcus*, or perhaps to a *Coenurus* bladder, and like these is undoubtedly to be referred to the six-hooked embryo. The first

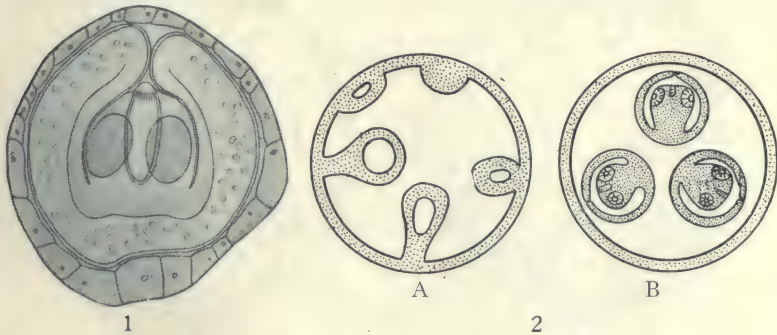


Fig. 1.—Cyst of *Monocercus didymogasteris*. (After Hill.)

Fig. 2.—Cyst of *Polycercus niloticus*. (Altered from Benham.)

developmental stage observed by Metchnikov appeared as a solid ball of about 0.08 mm. with an unusually thick cuticular envelope and cellular contents. The latter subsequently became clear on attaining a diameter of 0.14 mm. when the embryo lies on the inner surface of the cuticula in the form of a cellular layer. Soon the buds begin to form and at that exclusively from the cellular wall which becomes thicker at certain spots and sends little projections into the inner cavity. Although at first flat and connected by their broad bases with the cellular wall, the protuberances, as they grow larger, detach themselves from the subsequent layer. This separation is facilitated by the development of a hollow space in the interior of the basal portion, so that after a time the bud is only connected with the mother-bladder by a thin filament. Finally, this connection is destroyed and the bud thus becomes an oval body lying freely in the interior, so that at the end of its development the worm has exactly the same position as we formerly observed in *Cysticercus* (*Monocercus*) *arionis*.

From the above it will be seen that this type of budding is only an advance on the type described for *Monocercus*, in that more than one area of proliferation arises on the inner wall. These areas then hollow out and are later on detached when they become free in the central cavity of the parent cyst where each develops a head and becomes a *cysticeroid*. Haswell and Hill's type of *Polycercus* differs from the preceding type and will be dealt with later on.

3. *Coenurus* Rud.—In *Coenurus cerebralis* (Batsch) Rud. the stage is still further advanced than what occurs in *Polycercus* in that numerous scolices arise within the cavity of the parent cyst by a process of invagination of the cyst wall; but these never become detached from the cyst wall (Fig. 3).

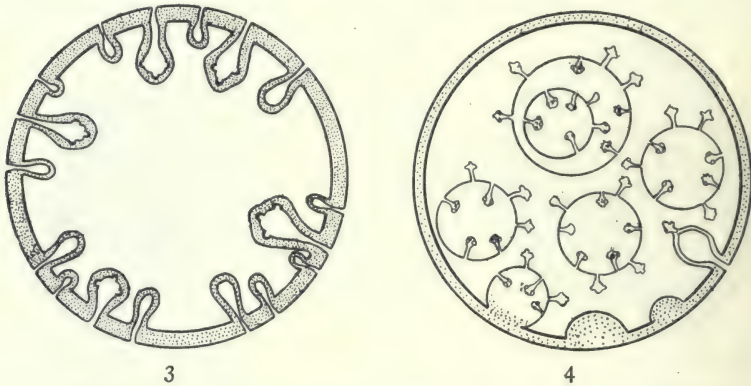


Fig. 3.—Cyst of *Coenurus cerebralis*. (From Benham.)

Fig. 4.—Cyst of *Echinococcus* sp. (From Benham.)

4. *Echinococcus* Rud.—Here the condition appears to be very much more advanced, combining some of the features seen in *Polycercus* and *Coenurus*. In the cyst of this genus secondary bladders are formed as proliferations from the inner wall of the parent cyst, exactly as in *Polycercus*, but instead of a single head or scolex being developed in each, a large number of scolices arise in each of these secondary cysts (Fig. 4). By a continuation of the same process, tertiary bladders may also be formed from the wall of the secondary bladders, whilst still enclosed in the parent cyst.

TYPE B.—WITH INTERNAL BUDDING IN AN UNKNOWN WAY

We may now consider two distinct types of endogenous budding in cysticercoids believed to be the larval stages of *Tetrarhynchus unionifactor* Herdman and Hornell, occurring in *Placuna placenta* Linn. and *Margaritifera vulgaris* Schum. (*Avicula fucata* Gould). One of these types was first recorded by Hornell (1906) in *Placuna placenta*.

It was recorded subsequently by Willey (1907) and the cysticercus was provisionally named *Merocercus*. Hornell described the formation of a single secondary cyst within the parent form, but Willey later on, working on the same form from the same locality, not only confirmed Hornell's discovery, but added that the endogenously produced larvae were a very common feature of this form and that multiple formation of endogens within a single cyst was likewise common. As many as twenty secondary cysts were seen in one parent cyst (Fig. 5). Monogenetic cysts were also observed by Willey (Fig. 6), but the multiple type of proliferation was the rule. This suggests that the monogen type may only be a stage in the development of the multiple type of cysts. This multiple endogeny, however, differs from what was recorded later on (Fig. 7) by one of us (Southwell, 1910) in that the parasite from *M. vulgaris* shows simple endogeny. Multiple



Fig. 5.—Cyst of *Merocercus* with a large number of daughter cysts. (After Willey.)

Fig. 6.—Monogen cyst of *Tetrarhynchus unionifactor* (?) *Monocercus*. (After Willey.)

Fig. 7.—Cyst of *Tetrarhynchus unionifactor*. (After Southwell.)

endogeny was never observed, though thousands of specimens were regularly examined at different seasons of the year over a period of six years.

The type observed by Willey is certainly more advanced than the one recorded by Southwell, even though the two have previously not been distinguished from one another.

As nothing is known regarding the mode of origin of these daughter endogens from the parent cyst we are unable to say anything regarding the relation of these forms with those described in Group A. The endogens may arise either as proliferations from the epithelial lining of the mother larval form, in which it would be similar to what occurs in *Monocercus*. On the other hand the daughter endogens may arise from the internal intima filling up the cavity of the mother larva. In that event, this method of endogenous budding would be quite

different from the other forms. It cannot, in any case, be parthenogenetic, as no eggs or egg-like structures are shown in the figures or described in the minute account of the anatomy of the larval form given by Herdman and Hornell (1906).

TYPE C.—WITH EXTERNAL BUDDING

In the following cases, budding takes place by proliferation from the external surface.

a. *Polycercus*.—A species of the genus *Polycercus* was found by Haswell and Hill (1894) in the earthworm *Didymogaster sylvatica* Fletcher. This species of *Polycercus* differed from the other species of this genus in having a definite type of development which consists of a process of external proliferation from the product of the hooked embryo in the following manner. "The hooked embryo in *Polycercus* develops into a rounded cellular body, which becomes enclosed in a cyst, probably entirely of an adventitious character. Buds are given

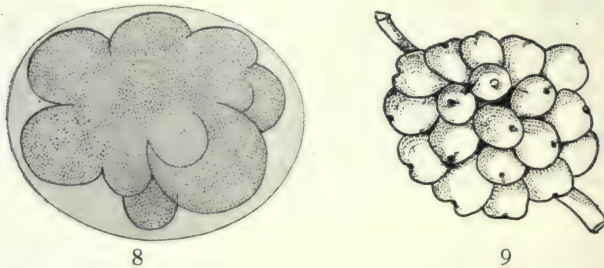


Fig. 8.—Cyst of *Polycercus* sp. (After Haswell and Hill.)

Fig. 9.—Cyst of *Staphylocystis glomeridis*. (From Benham.)

off from the periphery of the mass and develop into cysticercoids, which soon become free in the interior of the cyst (Fig. 8). The head, with its hooks and suckers is developed from the central portion of the solid body, the middle layers form the 'body' and the outermost, the caudal vesicle" (Haswell and Hill, 1894).

b. *Staphylocystis*. In the species *Staphylocystis glomeridis* Villot another type of asexual reproduction is to be met with. Here by the successive branching and external proliferation of secondary cysticercoids a complex organism is produced (Fig. 9). This type of external gemmation differs from that in *Polycercus* described above in that there is no external cyst wall in *Staphylocystis*, but as the cyst in *Polycercus* is considered by Haswell and Hill to be only an adventitious investment, the two may be considered to be nearly related.

c. *Sparganum*. In *Sparganum (Pleurocercus) proliferum* Ijima (1905), found in the skin of a Japanese woman, there is a definite

kind of budding from the external surface of the larval bothrioccephalid. In this case buds are given off from the parent stock in a more or less irregular manner (Fig. 10). The buds are direct outgrowths from the body of the larvae and later on they become detached. As many as seven larvae were found in the same cyst and were considered to be the detached buds.

d. *Urocystidium*. Beddard (1912), in examining parasites from *Fiber zibethicus*, found two tapeworms. These were considered by him to be the sexual and asexual phases of a new tapeworm. He

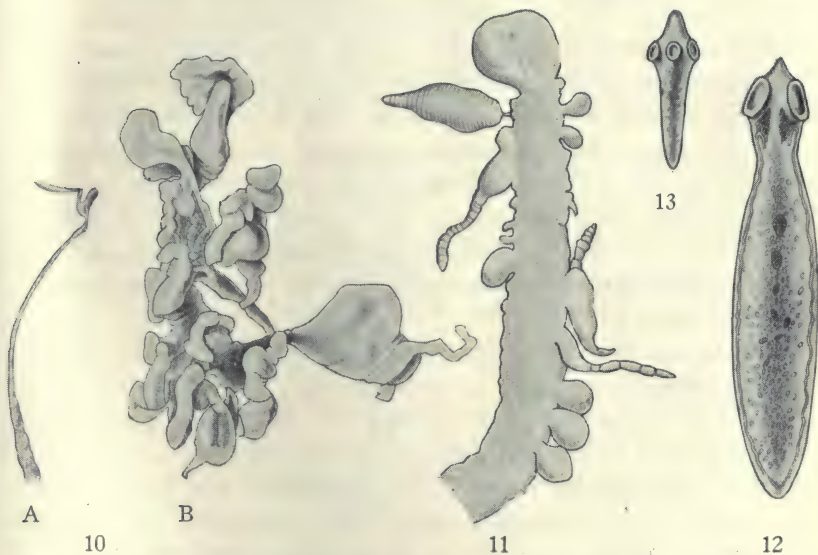


Fig. 10.—(a) A single individual of *Sparganum proliferum*. (After Ijima.)
 (b) A budding individual of *Sparganum proliferum*. (After Stiles.)

Fig. 11.—Asexual budding individual of *Urocystidium gemmiparum*. (After Beddard.)

Fig. 12.—An adult specimen of *Ilishia parthenogenetica* removed from the cyst. (Original.)

Fig. 13.—A young specimen of *Ilishia parthenogenetica* from the mesentery of Hilsa fish. (Original.)

regarded them as the type of a new genus which he named *Urocystidium*, the asexual form of which like *Sparganum* just referred to, buds off laterally and irregularly a series of young forms resembling the parent asexual form (Fig. 11). Beddard, however, considered his form differed from Ijima's type in that the buds were segmented. This does not appear to be an important difference, because Beddard's asexual worm is also segmented, whilst Ijima's *Sparganum proliferum* is unsegmented, and also the buds arising therefrom.

The above three types of external proliferation seem to be quite distinct from the various other types described in which buds arise from the inner walls of a cysticercoïd.

TYPE D.—WITH PARTHENOGENETIC REPRODUCTION.

We may now consider a method of parthenogenetic reproduction, which, as far as we are aware, is unique amongst the Cestoda and is only paralleled by what occurs in certain larval trematodes.

Ilishia parthenogenetica Southwell and Prashad, 1917.

This curious parasite was found in Bengal, India, heavily infecting the mesentery and liver of *Hilsa ilisha* (Ham. Buch), the Indian shad. In this case the worm is an adult, and so the parthenogenetic method of reproduction to be described, differs from all the preceding cases, which, as we have already noted, occur only in larval forms.

In this case definite egg-cells practically fill up the whole of the worm (Fig. 12). These eggs develop parthenogenetically into young forms which resemble the adult in all respects except size. After the worms (Fig. 13) have developed to the stage described above, the young forms find their way out of the parent form, become adult, and repeat the same life-history.

We do not propose considering here the metameric repetition of the proglottides in ordinary adult tapeworms, which by some authors is considered to be a type of budding. It will be obvious from the above facts that the methods of reproduction described are designed to ensure a very large infection for the propagation and preservation of the species—a very doubtful matter with animals having so complicated and uncertain a life-history as the forms described above. We are also of opinion that, up till now, too much attention has been paid to recording and describing new species of tapeworms, whilst in the vast majority of cases the life-histories have been utterly neglected. We are aware of the difficulty attending the elucidation of these life-histories, but it appears to us that labor in this direction would not go unrewarded and in all probability would result in the discovery of still other forms of reproduction and give results worthy of the labor and time. The field is wide and unexplored.

SUMMARY

In the above account we have discussed the followed methods of asexual and parthenogenetic reproduction amongst the Cestodes.

(1). Internal proliferation from the wall of the cysticercoïd, as seen in *Polycercus*, *Coenurus* and others.

(2). Endogenous budding, as seen in Willey's *Merocercus*.

(3). External budding, as exemplified in Haswell and Hill's species of *Polycercus*, *Staphylocystis*, etc.

(4). Parthenogenetic reproduction in *Ilisia parthenogenetica*, an adult tapeworm of doubtful affinities.

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THE EXCRETORY SYSTEM OF *AGAMODISTOMUM MARCIANAE* (LA RUE), THE AGAMODISTOME
STAGE OF A FORKED-TAILED
CERCARIA *

WILLIAM WALTER CORT
University of California

In a recent paper La Rue (1917) described as *Cercaria marcianae* a new larval trematode from *Thamnophis marciana* (Baird and Girard). Since this form is in the agamodistome stage, the species should be referred to the provisional genus *Agamodistomum* Stossish and the name changed to *Agamodistomum marcianae*. In the summer of 1915 I collected material of this same species from the tissues and lymph spaces of tadpoles of *Rana pipiens* and *Rana clamitans*, and from the digestive tract and body cavity of the garter-snake, *Thamnophis sirtalis*, from the region of Douglas Lake, Michigan. It was found possible to introduce these larvae into the snakes by feeding them with infected tadpoles, but no advance in development followed, and the larvae soon made their way out from the intestine into the body cavity and tissues. This observation and La Rue's description of *Agamodistomum marcianae* from a snake show that this species has two secondary intermediate hosts. Since tadpoles formed a very large part of the food of the garter-snakes examined, it seems very probable as suggested by La Rue (1917:8) that the snakes obtain their infection from this source. This makes a very unusual complication in the life-history of this trematode involving a change of host without an advance in development. I have also found *Agamodistomum marcianae* in lymph spaces under the skin of adults of *Rana pipiens* from North Judson, Indiana.

The host in which *Agamodistomum marcianae* completes its development is not known. Also its structure at this stage gives little clue to the systematic position of the adult. The character of its cephalic glands and excretory system, however, indicates that it has developed from a forked-tailed cercaria, and a comparison of its structure with that of *Cercaria emarginatae* Cort and *Cercaria douglasi* Cort (see Cort, 1917) shows such close correspondence in the structure of the digestive and excretory systems and in the characteristics of the spination, suckers and cephalic glands that a very close relationship is established. Differences from *Cercaria emarginatae* in the number of cephalic glands and from *Cercaria douglasi* in the structure of the excretory bladder makes it impossible to connect *Agamodistomum marcianae* with either of these species. I should expect, however, to find the cercaria of this species to be very much like these two cercariae.

* Publication No. 41 from the University of Michigan Biological Station.

Except for the arrangement of the cuticular spines my studies on *Agamodistomum marciae* agree with La Rue's description. I am able to add to his account a complete analysis of the excretory system, made from the study of large numbers of living specimens. The excretory system of this species is of especial significance since it gives an idea of the development of the type of excretory system found among the forked-tailed cercariae.

La Rue's (1917:4) account of the arrangement of the cuticular spines in *Agamodistomum marciae* is as follows: "The surface of the body is covered with minute spines arranged in regular longitudinal rows. The spines at the anterior end of the body are a trifle longer

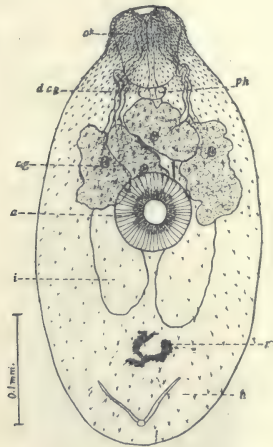


Fig. 1.—Camera lucida drawing of *Agamodistomum marciae* (La Rue), ventral view; *os*, oral sucker; *ph*, pharynx; *a*, acetabulum; *d cg*, ducts of cephalic glands; *cg*, cephalic glands; *i*, intestinal cecum; *b*, excretory bladder; *r*, primordia of reproductive organs.

than elsewhere." My studies on the arrangement of the spines in living specimens of this species and from toto mounts of my own material and of material sent me by La Rue make necessary a revision of his account. The ventral surface is completely covered with spines which are very thickly set over the anterior tip and somewhat scattered in the postacetabular region. The margin of the acetabulum is armed with two to three rows of closely set spines pointing in, which are so placed that they add greatly to the gripping power of the sucker. The dorsal surface has the same distribution of the spines as the ventral to the region of the bifurcation of the intestinal caeca, but back of this level the cuticula is smooth except for a few scattered spines near the posterior tip. Figure 1 shows the distribution of spines on the ventral surface of *Agamodistomum marciae*.

In the description of the excretory system of *Cercaria marcianae* the names of the subdivisions as used by Looss (1894:156) will be employed. This writer divides the trematode excretory system into four main subdivisions which he considers to be natural and recognizable in all forms. They are (1) the excretory vesicle or bladder; (2) the collecting tubes; (3) the capillaries, and (4) the flame cells. The bladder is the region next to the excretory pore, and is the only part of the system which has a definite cellular lining and muscle layers. The collecting tubes connect the bladder with the capillaries. The collecting tubes which flow directly into the bladder, which for convenience may be called the main collecting tubes, are often divided

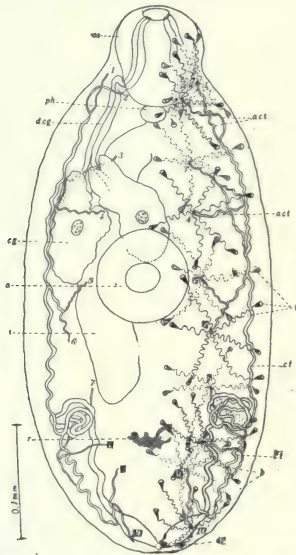


Fig. 2.—Excretory system of *Agamodistomum marcianae* (La Rue), ventral view. On the right side of the figure all parts of the excretory system are shown, but on the left side the capillaries and flame cells are omitted. The numbers 1 to 10 on each side indicate the points where the accessory collecting tubes are joined by the capillaries; letters as before; also, *act*, accessory collecting tubes; *f*, flame cells; *ct*, main collecting tubes; *e*, excretory pore.

and subdivided, a reduction in caliber following division. For convenience also the collecting tubes are divided by Looss into the principal collecting tubes and the accessory collecting tubes. The latter include those which are directly connected with capillary groups. The capillaries are the tubules from the flame cells, and are usually arranged in groups of a definite number.

Figure 2 shows the excretory system of *Agamodistomum marcianae*. On the right side of the figure all parts of the system are shown, while on the left side the flame cells and their capillaries are omitted. The numbers 1 to 10 on each side indicate the points where the accessory collecting tubes are joined by the groups of capillaries.

The excretory pore (*ep*) is at the posterior end slightly dorsad in position and forms the only point of union of the lateral halves of the system. The bladder (*b*) is V-shaped, the sides extending about half way up to the acetabulum, where they form complicated coils. Near the pore the bladder on each side is dilated for a short distance. It is in these dilated portions that the contraction and expansion having to do with the expulsion of fluid is most noticeable. Each side of the bladder receives an anterior and posterior main collecting tube (*ct*). Each anterior main collecting tube receives three branches each of which is divided into two accessory collecting tubes (*act*), while each posterior main collecting tube receives two branches each of which is divided into two accessory collecting tubes. This makes a total of five pairs of accessory collecting tubes on each side (numbers 1 to 10). Each of these accessory collecting tubes receives the capillaries from six flame cells. One accessory collecting tube of each pair with its group of capillaries is dorsal in position and the other is ventral. In

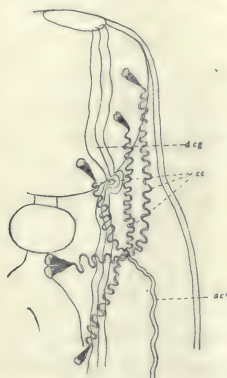


Fig. 3.—Group of flame cells and capillaries at the anterior end of *Agamodistomum marcianae* (La Rue), showing a flame cell in the process of longitudinal fission; letters as before.

Figure 2 the capillaries and flame cells of the dorsal side are shown with dotted lines. There is then a total of one-hundred and twenty flame cells, connected by their capillaries in groups of six with ten pairs of accessory collecting tubes. The only variation from the pattern just described was found in the capillary group shown in Figure 3, in which one of the accessory collecting tubes of the anterior end is joined by only five capillaries. At the end of one of these capillaries was a flame cell apparently in the process of longitudinal fission. This observation is suggestive of a possible method of formation of capillary groups. Altho I searched carefully for further evidence on this point no other instances of such division were found.

The comparison of the excretory system of *Agamodistomum marcianae* with that of the closely related forked-tailed cercaria *Cercaria emarginatae* (Cort, 1917, Fig. 2 B), gives an idea of the method of

development of this type of excretory system. The general pattern of the two systems is the same, but the subdivisions are much more complicated in the agamodistome. The divisions of the main collecting tubes correspond in number and position in both forms. In *Cercaria emarginatae* these ten subdivisions end in flame cells and are therefore equivalent to capillaries, while in *Agamodistomum marcianae* with increased body size there is need of an increased number of flame-cells, and the ten divisions of the main collecting tubes are bifurcated into twenty accessory collecting tubes, each receiving the capillaries from six flame-cells. Since *Agamodistomum marcianae* does not belong to the same species as *Cercaria emarginatae* it cannot be argued that its excretory system is necessarily derived from one exactly like that of this cercaria. Yet the structural agreement between these two species indicates such close relationship and the homologies of their excretory systems are so striking, that when the conservativeness of the trematode excretory system is considered, it seems certain that the above comparison shows in a general way what is to be expected in the development of the type of excretory system of the forked-tailed cercariae. It is interesting to note that these observations agree in with the theory of Looss (1894; 248-251) as to the method of development of the trematode excretory system. However, the total available data on the whole subject of the development of the trematode excretory system is so limited that any adequate attempt to establish general principles must await an increase in knowledge.

SUMMARY

1. The larval trematode described by La Rue from *Thamnophis marcaiana* as *Cercaria marcianae* should be named *Agamodistomum marcianae*.
2. The excretory system of this species is very complicated consisting of sixty flame cells on each side arranged in a very definite pattern.
3. The finding of a flame cell in one of the groups dividing by longitudinal fission suggests that the capillary groups may be formed by such divisions.
4. *Agamodistomum marcianae* is the agamodistome stage of a forked-tailed cercaria, and its excretory system gives an idea of the development of the excretory system in this group.

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NATURAL OCCURRENCE OF EOSINOPHILIAS

S. HADWEN

When the juices obtained from *Hypoderma* larvae, either diluted or otherwise, are injected under the skin of a susceptible animal, the first effect noticed will be local. At the point of injection a blister-like spot will be seen, and a necrotic area will occur. In twenty minutes to half an hour, swelling will be noted. The necrotic spot in the center will be depressed. Smears made from the swelling some hours later, reveal the presence of an eosinophilia, and if the material which was injected contained bacteria, phagocytosis by the eosinophils. This observation of phagocytosis by the eosinophils was made by myself and certified by Dr. B. H. Ransom at Washington, D. C. The reason for the eosinophils assuming the rôle which is usually assigned to the neutrophils, is apparently because the bacteria are rendered attractive by their being bathed in the verminous juices. These cells containing bacteria are not numerous, and were only found in a few cases. According to Weinberg and Séguin (1914, 1915), in their reports on the experimental injections of the juices of *Ascaris* and other intestinal parasites, the principal function of the eosinophils is to neutralize toxins, but they also ingest small particles of debris or bacteria when they are present in verminous fluids. They were also successful with mixtures in vitro. These findings seem to indicate that the eosinophils play a very important rôle in picking up the bacteria which are introduced into the body by the bites of intestinal parasites, and in neutralizing toxins which may be absorbed. The swelling which follows these injections may persist for several days. The same effect may be produced by puncturing or crushing a warble larva "in situ" under the skin. The liberation of the proteins contained in the grub causes a local swelling, anaphylactic in type and stimulate an eosinophilia. When a smear is made from the contents of the swelling, large numbers of eosinophils are seen and innumerable granules. Proof that the eosinophils are attracted by the secretions and excretions of warble larvae alone and not by bacteria, can be found by examining the fluids in the edemas surrounding them in the gullet. Here the larvae are usually sterile as I have proved by examining numerous slides, also by extracting the grubs and incubating them in bouillon. Three out of four grubs thus extracted proved to be sterile. Further, the grubs which were found in the neutral canal in a

number of other animals (7 in one case) must also have been sterile, as only small edematous tracks and gray areas in the fat were noted. In the gullet the eosinophil was the principal cell encountered. There were only a few neutrophils and a smaller percentage of mononuclears and macrophages. Pus was taken from the cavities beneath the skin of cattle in which warble larvae were living. Eighteen cows were examined and in only two instances did I find bacteria in large numbers. In one of these, it was only after a prolonged search that an eosinophil was found. In the other, only an occasional one. In the sixteen other smears eosinophils were plentiful and bacteria correspondingly scarce. Differential counts were attempted, but of course, only give a rough idea of the percentage owing to the degenerated condition of many of the cells. In one case the neutrophils were 75%, the mononuclears 12.5%, the eosinophils 7.5% and the macrophage cells 4.4%. On the same date the blood taken from the general circulation showed neutrophils 25.75%, the mononuclears 68% and the eosinophils 6.25%.

No doubt the large percentage of the neutrophilic leukocytes in the pus smears was due in part to the presence of bacteria, as in the swellings of the gullet they were only found in small numbers.

The percentage of the eosinophils in many of the other cases was not as high as in the example given and in one of them it was as low as 2%. If, however, there had been no bacteria with which to contend, it is probable that the percentage would have been higher owing to the presence of fewer neutrophils.

Another experiment might be cited to prove that the eosinophile cell is *par excellence* the agent which truly repels and neutralizes verminous products. A few drops of warble juice were instilled into a steer's eye at 10 a. m., and at 7:30 p. m. smears were made from the discharge. The cells were practically all eosinophils.

These experiments are a continuation of the work recorded by E. A. Bruce and myself in 1916, relating to the injection of juices derived from oestrid larvae, and of later experiments made in collaboration with Dr. B. H. Ransom at Washington, D. C. Nearly all the publications to which I have had access, discussing injections of verminous juices, have reference to those inhabiting the intestines, and though the fluids from the parasites may have been rendered sterile by filtration, their proteins may have become mixed during the process of absorption or ingestion of the intestinal contents by the parasites (the hydatid cyst fluids excepted). *Hypoderma* larvae are very suitable for such experiments, seeing that they are sterile until they bore through the skin, and they nourish themselves solely on animal tissues. It is a well-known fact that, as a rule, in verminous anemias the percentage of eosinophils is high, but in some instances they may be very scarce. Weinberg and Séguin noted this in their experiments and suggest that the few eosinophils which were in the

circulation had been attracted to the parts affected in order to repel a verminous invasion.

Experiments are contemplated to determine what curative effects the injection of worm juices may have on such cases.

I am indebted to Dr. Ransom for sending me two important papers recently, by Marchesini and Barnett which bear on this subject.

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THREE UNUSUAL CASES OF PARASITISM (A SLUG, A MYRIAPOD, AND COCKROACHES) REPORTED IN MAN

C. W. STILES

Professor of Zoology, U. S. Public Health Service

Cases of pseudoparasitism and of spurious parasitism occasionally come to the attention of medical zoologists. Some of these are reduced to errors in observation, some to the chance presence of an organism in a host, some to attempts on the part of hysterical patients to mystify their physicians.

The three following cases are rather unusual and are published on this account.

Spurious parasitism due to a slug (*Limax flava*). Specimen 11, 165. A slug, determined by Dr. Paul Bartsch as *Limax flava*, was sent to the Hygienic Laboratory by the Maryland State Department of Health with the report that it was alleged to have been passed from the bowels by a patient in Baltimore.

As it is in good state of preservation it is difficult to assume that it passed through the intestinal canal.

A geophilid myriapod from stomach. No. 11, 159. Mr. L. M. McCormack of Ashville, N. C., has recently sent in a myriapod with the following history:

"I am sending you a specimen which the physician who brought it into the laboratory declares was in the stomach contents, brought up by a stomach pump; it looks to me like an adventurer and not an intestinal parasite."

Dr. L. O. Howard has determined this organism as a geophilid.

Several cases of the presence or alleged presence of myriapods in man have been recorded and in most instances the organisms have been geophilids. In some cases they were reported from the nasal passages, and associated with severe headache, in others they were reported from the intestine. R. Blanchard has summarized cases recorded by Littre (1708), Kerckring (1717), Sandifort (1789), Blumenbach (1807), Scoutetten (1827), Lefèvre (1833), Laboulbène (1867), Le Roy (1878), and Giard (1880).

Croton bugs (*Blatella germanica*) said to have come from an abscess of the jaw. Specimen 11, 226. This specimen was forwarded by Dr. Harold N. Cole of Cleveland, Ohio, with the following information:

Patient, a luetic in the City Hospital, had a large horse-chestnut sized swelling under angle of left jaw; the lesion has been very tender and the glands posterior to this are swollen and tender and scars of other glands are also seen under the jaw. On Nov. 21, 1916, patient suddenly had a chill with a temperature of 103 and began to expectorate a bloody sputum; upon examination of this expectoration a capsule was found together with some small arthropods. "Of course we cannot be absolutely sure, but apparently the patient expectorated these organisms."

The organisms in question were determined by A. N. Caudell of the U. S. Bureau of Entomology, as young cockroaches; the capsule was probably the egg case of the cockroach.

NOTES

Dr. C. A. Kofoid, Consulting Biologist for the California State Board of Health, in the Bureau of Communicable Diseases, has been granted leave of absence for war work. Dr. W. W. Cort has become acting Consulting Biologist in charge of the Biological Division during Professor Kofoid's absence.

The Severance Union Medical College at Seoul, Korea, which was established as a special school on May 14, 1917, includes in its plan of organization a Research Department under the direction of Dr. Ralph G. Mills. Prominent among its aims as listed in the report of the director stands, "To investigate botanical and zoological problems, especially those that bear upon the questions of animal parasites and native drugs."

The National Research Council has asked the persons named below to serve as a committee on medical zoology which will be related on the one hand to its work in zoology and on the other hand and more especially to its work on medicine:

ENTOMOLOGY

Dr. L. O. Howard, Department of Agriculture, Washington, D. C. (chairman of group); Prof. Charles T. Brues, Bussey Institute, Forest Hill, Mass.; Prof. C. V. Riley, Cornell University, Ithaca.

PROTOZOLOGY

Prof. (Major, S. C. N. A.) C. A. Kofoid, University of California, Berkeley, Calif. (chairman of group) (Fort Sam Houston, Texas); Dr. Theobald Smith, Rockefeller Institute, Princeton, N. J.; Prof. F. G. Novy, University of Michigan, Ann Arbor.

HELMINTHOLOGY

Prof. Henry B. Ward, University of Illinois, Urbana (chairman); Dr. C. W. Stiles, U. S. Public Health Service, Washington; Dr. Allen J. Smith, University of Pennsylvania, Philadelphia.

The war organization of the National Research Council includes a division on Medicine and related sciences of which Dr. Richard M. Pearce, a member of the Council, is chairman. The committee on medical zoology is a section of this division.

BOOK REVIEWS

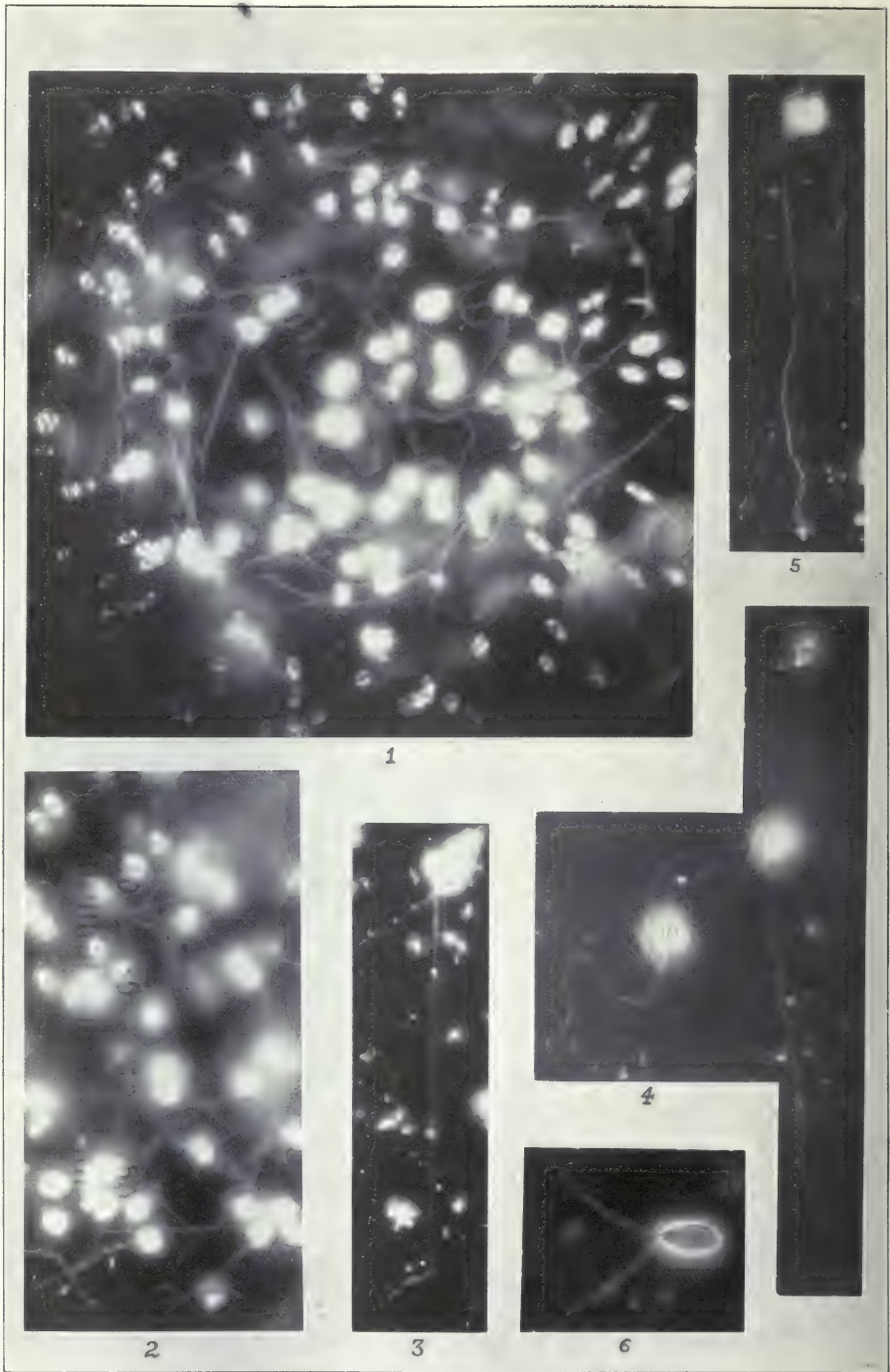
THE CLINICAL PATHOLOGY OF THE BLOOD OF DOMESTICATED ANIMALS. Samuel Howard Burnett, A.B.M. M.S., D.V.M. Professor in Comparative Pathology, New York State Veterinary College, Cornell University, Ithaca, New York. The Macmillan Company, 1917. xvi + 166 pages. 4 plates, 23 figures. \$2.25.

The author has attempted to do for students and workers in the veterinary field what numerous other works have done for the human subject. This treatise, which is a pioneer in its field, has undergone considerable change in this, the second edition. As a text for college work it fills a conspicuous need of undoubted value and deserves commendation.

Two chapters deal with topics of especial interest to students of animal parasites. In Chapter IX, Infectious Diseases Due to Protozoa, the author discusses spirochetosis, Texas fever, various forms of piroplasmosis and of trypanosomatoses. In each case the problem of changes in the blood is carefully analyzed and interpreted in so far as their significance can be determined.

The last chapter, Chapter XI, Diseases Due to Animal Parasites, deals with the metazoan parasites alone. It seems unduly brief and has hardly received attention commensurate with its importance or proportionate to the general plan of the book. It must be confessed that in this respect the author merely follows the habits of most workers on human hematology. The topic is deserving of more extended treatment in a later edition.

Under the heading of Hookworm Campaigns the *Tropical Diseases Bulletin* for February 14 (vol. 11, p. 100-112) presents an extremely valuable summary of operations which were originally reported in scattered and often inaccessible publications. Reviews of this character call for special comment even though all the work done by the *Bulletin* has been organized in most effective fashion and deserves the highest praise from scientific workers at home and abroad.



EXPLANATION OF PLATE

Figs. 1-5.—Spores of *Nosema bombycis* with extruded polar filament after subjection to the action of perhydrol.

Fig. 6.—A spore of *Myxosoma funduli* treated in the same way.

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EXPERIMENTS ON THE EXTRUSION OF POLAR FILAMENTS OF CNIDOSPORIDIAN SPORES *

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It has been generally believed that when a Cnidosporidian spore is introduced into the digestive tract of a new host, the first change of the spore prior to the germination of the sporoplasm is the extrusion of its polar filament, according to the observations made by Thélohan (1895), Stempel (1909), Fantham and Porter (1912), Kudo (1916), and others. Osmotic pressure is considered in this case to be the cause of the filament extrusion.

In the case of the artificial cultivation of the Cnidosporidia, starting with the spore-stage, therefore, the first observation should be concentrated on the filament. From this point of view I have again taken up the question of the filament-extrusion, which I partially worked out about four years ago (Kudo, 1913).

Since Balbiani discovered in some Myxosporidian spores the presence of a polar filament which was extruded from the spore under the action of alkalis, several investigators have tried a large number of reagents in the effort to produce extrusion of the filament of Cnidosporidian spores of various species. The reagents used and the authors' names are given in Table 1.

Most authors agree that the percentage of spores which extrude the filament when subjected to the action of the above-mentioned reagents is very small and irregular. It seems quite remarkable that these reagents should affect only certain particular species, as would appear from Thélohan's experiment (1895).

In the case of mechanical pressure, I could see almost all of the spores extruding their filaments at places where the pressure was apparently strong enough to drive the filament out. When the spore material is mixed with comparatively coarse particles of tissue, however, the pressure method does not give uniform results.

* Work carried out in the laboratories of Dr. Noguchi and under his direction.

Frey and Lebert and Haberlandt and Verson studied the action of several chemicals upon the spore of *Nosema bombycis* without noticing the filament extrusion.

Of the many chemicals which I have tried up to the present, hydrogen peroxid, as first found by Dr. Noguchi, has proved best. Following is a brief summary of the experiments with this reagent, which have been carried out chiefly on *Nosema bombycis*.

TABLE 1

Reagents	Author's Name and Parasite Used
Acetic acid	Thélohan (<i>Myxosoma, Henneguya</i>), Wasielewski, Fantham and Porter (<i>Nosema apis</i>)
Ether	Thélohan (<i>Thelohania</i>) Gurley, Pfeiffer, Wasielewski, Auerbach
Alkalies (KOH and NaOH)	Balbani, Thélohan, Wasielewski, Auerbach, Kudo
Ammonia	Auerbach, Schuberg (<i>Pleistophora longifilis</i>)
Boiling water	Gurley, Wasielewski, Auerbach
Distilled water	Thélohan (<i>Chloromyxum</i>), Wasielewski, Auerbach
Glycerin	Schneider, Gurley, Wasielewski, Auerbach, Awerinzew (<i>Myxobolus magnus</i>)
Iodin alcohol	Stempell (<i>Thelohania mülleri, Nosema anomalum</i>)
Iodin water	Thélohan (<i>Ceratomyxa, Glugea, Pleistophora</i>), Léger, Wasielewski, Auerbach, Fantham and Porter
Mineral acids:	
Hydrochloric acid	Thélohan (<i>Thelohania</i>), Gurley, Wasielewski, Auerbach
Nitric acid	Thélohan (<i>Myxosporidia, Glugea bombycis, Thelohania</i>), Gurley, Pfeiffer (<i>Thelohania mülleri</i>), Wasielewski
Sulphuric acid	Bütschli, Thélohan (<i>Sphaeromyxa</i>), Gurley, Wasielewski, Auerbach, Awerinzew
Physiological solution	Lacépède (<i>Pleistophora macrospora</i>)
Pressure	Gurley, Auerbach, Kudo

The sterile silk-glands taken from the highly infected larvae of *Bombyx mori* furnished the original material for the experiment. They were emulsified with sterile water as well as with sterile Ringer's solution, kept in small test tubes in the refrigerator at a temperature of 6° C., and taken out from time to time for examination. An equal quantity of a strong solution of hydrogen peroxid and the emulsion were mixed either on a slide or in a test tube and examined microscopically. Observations were usually made under the dark field microscope, which, though it seems to have been neglected in this field of protozoology, affords a quick and entirely suitable means of carrying out the necessary observations. The permanent preparation was made according to Löffler—Kudo's method (1913). Fontana's staining for *Treponema pallidum*, however, was found to be equally good.

When a drop of the emulsion of *Nosema bombycis* is mixed on an acid-free slide with a drop of perhydrol,* active bubbling takes place. By examining the preparation under the dark-field microscope, almost all of the spores are seen to shoot out the polar filament with great rapidity (see plate). The phenomenon takes place immediately and lasts for several minutes.

The emulsion in Ringer's solution seems to give a more vigorous extrusion than the plain water emulsion.

* About 30% H₂O₂ by weight, Merck.

A weak alkalin reaction of the emulsion as rendered by the addition of sodium bicarbonate seems to accelerate the extrusion of filaments, while acidulation inhibits extrusion.

That a concentrated solution of perhydrol is much more effective than a weaker one is shown in Table 4.

An attempt was made to induce the extrusion of polar filaments with the emulsions prepared from old dried materials, but it was found that no extrusion took place with such specimens. Apparently the desiccation of the spores rendered them insensitive to the extruding action of the reagent. The results of the experiments made with freshly dried materials were identical with those obtained with the old specimens and are shown in Table 5. An electric fan was employed to dry the emulsion during the first 24 hours, after which the slides with the dried emulsions were left at room temperature.

TABLE 2

	Immediately After Treatment	After One Hour	After 24 Hours
Water emulsion, 1 c.c. + perhydrol, 2 c.c.	Many extruded filaments	Fairly many extrusions	Many filaments detached
Ringer emulsion, 1 c.c., + perhydrol, 2 c.c.	Almost all spores show filament	Almost all spores show filament	Many filaments detached

TABLE 3

	Control	NaHCO ₃ (1%)	HCl (1%)
Ringer emulsion, 1 c.c., + perhydrol, 2 c.c.	Many filaments extruded	Almost all spores show filament	No extrusion

TABLE 4

Ringer emulsion, 1 c.c. + perhydrol (30%), 1 c.c.	Immediately after the treatment great many extruded filaments
Ringer emulsion, 1 c.c. + perhydrol solution (3%), 1 c.c.	A few extruded filaments

TABLE 5

	Perhydrol and spores in alkalized emulsion	1% aqueous emulsion of the fresh tissue, NaHCO ₃ and perhydrol	Spores taken from dried moths, which were kept over one year, pressure method
Immediately after the aqueous emulsion was made	Almost all of the spores show extruding filaments	Vigorous bubbling	Many filaments
Dried for 2 hours on slide	Fairly many extruded filaments	Bubbling less active	
Dried for 16 hours on slide	Many extruded filaments	Weak bubbling	
Dried for 24 hours on slide	A few detached filaments (?)	Only few bubbles	
Dried for 3 days on slide	No extrusion	No bubbling nor bubbles	
Dried for 5 days on slide	No extrusion	No bubbling nor bubbles	
Dried for 10 days on slide	No extrusion	No bubbling nor bubbles	

It is noteworthy that the number of extruded filaments progressively diminished as the desiccation went on, until, after three days, the phenomenon ceased to occur. With regard to the mechanism of extrusion by means of perhydrol, it seems probable that a physical force, comparable to that produced by pressure, develops within the spores and expels the polar filament. This force may be none other

than the gas evolved through the decomposition of hydrogen peroxid by the peroxydase contained within the spores. In support of this view it may be cited that the spores preserved for more than one year still extruded their filaments when subjected to mechanical pressure. The failure of the dried spores, therefore, to extrude the polar filament under the action of perhydrol is a sign of the weakening or absence of the peroxydase, but bears no relation to the viability of such spores.

TABLE 6

	Perhydrol + spores in alkalized emulsion	Pressure
1 per cent. methyl alcohol	Filament extrusion.....	Filament extrusion
3 per cent. methyl alcohol	Filament extrusion.....	Filament extrusion
10 per cent. methyl alcohol	Filament extrusion.....	Filament extrusion
30 per cent. methyl alcohol	Filament extrusion.....	Filament extrusion
60 per cent. methyl alcohol	Few extruded filaments....	Few extruded filaments
70 per cent. methyl alcohol	Few detached filaments (?)	?
85 per cent. methyl alcohol	No extrusion	No extrusion
Absolute methyl alcohol...	No extrusion	No extrusion
Control without alcohol treatment	Almost all spores show extruded filament	Many extruded filaments

TABLE 7

	Perhydrol + spores in alkalized emulsion	Pressure
10% ethyl alcohol, 16 hours	Few extruded filaments....	Many extruded filaments
30% ethyl alcohol, 16 hours	Few extruded filaments....	Few extruded filaments
34% ethyl alcohol, 16 hours	Few extruded filaments....	?
38% ethyl alcohol, 16 hours	No extruded filaments....	?
50% ethyl alcohol, 16 hours	No extruded filaments....	No extruded filaments
80% ethyl alcohol, 16 hours	No extruded filaments....	No extruded filaments
Absolute methyl alcohol...	No extruded filaments....	No extruded filaments
Control without alcohol treatment	Almost all spores show extruded filament	Extruded filaments in many places

TABLE 8

	Ethyl Alcohol +1% NaHCO ₃ (0.5 C.c.) +H ₂ O ₂ (0.02 C.c.)		Methyl Alcohol +1% NaHCO ₃ (0.5 C.c.) +H ₂ O ₂ (0.02 C.c.)	
	Vigorous bubbling	Vigorous bubbling	Vigorous bubbling	Vigorous bubbling
Control without alcoholic treatment				
Emulsion +10%	Active bubbling	Active bubbling	Active bubbling	Active bubbling
Emulsion +20%	Active bubbling	Active bubbling	Active bubbling	Active bubbling
Emulsion +30%	Active bubbling	Active bubbling	Active bubbling	Active bubbling
Emulsion +40%	Active bubbling	Active bubbling	Less active bubbling	Active bubbling
Emulsion +50%	Active bubbling	Active bubbling	Less active bubbling	Active bubbling
Emulsion +60%	Less active bubbling	Active bubbling	No bubbling	Less active bubbling
Emulsion +70%	Less active bubbling	Active bubbling	No bubbling	Much less active bubbling
Emulsion +80%	No bubbling	Less active bubbling	No bubbling	No bubbling
Emulsion +90%	No bubbling	No bubbling	No bubbling	No bubbling
Emulsion + absolute	No bubbling	No bubbling	No bubbling	No bubbling

It is evident that in the case of perhydrol, the extrusion of filaments is produced by a positive pressure created within the spores, but it was not shown whether or not a negative pressure by means of a vacuum will accomplish the same effect. For this reason, fresh *Nosema* spores were placed in a vacuum jar from which the air was exhausted with an electric vacuum pump. A vacuum at 2 mm. of mercury maintained for one hour did not expel the polar filaments from the spores.

With reference to the effect of alcohol upon the extrusion of polar filaments, it may be remarked here that Thélohan (1895) working with various chemicals as already quoted in the earlier part of this article, failed to obtain any extrusion with several Myxosporidia preserved in alcohol. On the other hand, Gurley (1894) reports that a rather small proportion of the spores of certain Myxosporidia preserved in alcohol extruded the polar filament under the action both of sulphuric acid and iodine water.

An experiment bearing on this point was made in the present study with *Nosema bombycis*. The technic used to test the action of alcohol upon the *Nosema* spores consisted in mixing 0.5 c.c. of a water emulsion and 3 c.c. of each of several alcohols in test tubes and allowing the mixtures to stand for ten minutes, inclusive of the time required for centrifugation, or, in some instances, for 16 hours at room temperature before centrifugation. The spores deposited at the bottom of the centrifuge tubes were subjected to the action of perhydrol in concentrated form. The results are recorded in Table 6 and 7.

As will be noted in the foregoing tables, of the spores subjected to the action of 60 per cent. methyl alcohol for 10 minutes, or of 34 per cent. ethyl alcohol for 16 hours, a few still extrude their polar filaments when treated with perhydrol. No polar filament is seen to be extruded from the spores treated with 85 per cent. methyl alcohol for 10 minutes or 30 per cent. ethyl alcohol for 16 hours. Methyl alcohol weaker than 30 per cent. allowed to act for 10 minutes had much less effect upon the phenomenon. Spores which have remained in 30 per cent. ethyl alcohol for sixteen hours were rendered almost insusceptible to the extruding action of perhydrol, and the filament extrusion became less as the percentage of alcohols rose, until, when the spores were treated with strong alcohol for a sufficient length of time, no filament escaped from the spore. In this instance, as in that of desiccation, the reduction of susceptibility of these spores to the extruding action of perhydrol may be explained by assumptions similar to those advanced for the effect of desiccation. Alcohol may have altered the permeability of the spores, or possibly the elasticity of the polar filament is reduced by the hardening action of alcohols, rendering it unable to unfold for protrusion. Another factor is the gradual destruction of the peroxydase as the concentration of alcohols is increased. Table 8 records the results of an experiment which shows that the peroxydase is considerably damaged by 30 to 40 per cent. methyl alcohol or 50 to 60 per cent. ethyl alcohol when acted upon for three hours. Thus 0.05 gm. of a fresh rabbit's kidney was emulsified with 5 c.c. of distilled water; 0.05 c.c. of the emulsion was

mixed with 0.5 c.c. of each of several grades of alcohol, and the mixtures were left in the refrigerator at 6° C. for three hours. At the end of this period they were further treated with perhydrol (0.02 c.c.), or with 1 per cent. NaHCO_3 (0.05 c.c.) and perhydrol (0.02 c.c.). The results were as follows:

It may be concluded, therefore, that a fresh tissue treated with 90 per cent. ethyl alcohol or 80 per cent. methyl alcohol is no longer able to split hydrogen peroxid under the experimental conditions described above. Assuming that the extrusion of polar filaments is caused by the decomposition of H_2O_2 by a minute amount of the intracellular ferment (peroxydase), it is not difficult to understand why the extrusion becomes less certain when desiccation or alcohol treatment is applied to the spores, since there results an inactivation of the ferment sufficient to cause the occurrence of the phenomenon.

Spores of several species of Myxosporidia were also subjected to the action of perhydrol. The following Myxosporidian spores, according to my observations (Kudo, 1916a) when treated with this reagent, extrude their filaments: *Myxidium* sp.; *Zschokkeella acheilognathi* Kudo; *Myxosoma funduli* Kudo (Fig. 6).

SUMMARY

1. A concentrated solution of hydrogen peroxid is the most perfect and convenient reagent for producing extrusion of the polar filament from spores of *Nosema bombycis* and of some Myxosporidia in the fresh state.
2. The action of hydrogen peroxid is accelerated by the presence of weak alkalies.
3. Ringer's solution emulsion is more favorable for filament extrusion than water emulsion.
4. The action of hydrogen peroxid in extruding the polar filament is less effective upon spores which have been desiccated at room temperature than upon fresh ones. Spores dried on a slide for three days do not extrude the filament.
5. The pressure method gives, generally speaking, the same results as the perhydrol method, except that it produces fewer examples of extruded filament.
6. A spore emulsion centrifuged with 60 per cent. methyl alcohol for 10 minutes or mixed with 34 per cent. ethyl alcohol for 16 hours shows filament extrusion under the action of perhydrol.

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TWO NEW CYSTOCERCOUS CERCARIAE FROM NORTH AMERICA*

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Following the description of the unique anchor-tailed *Cercaria mirabilis* by Braun (1891), Ward (1916) published an account of two similar American cystocercous species, *Cercaria wrightii* and *C. anchoroides*. The former of these American species had been previously mentioned by Wright (1885) and Leuckart (1886). Knowledge of the structure and relationship of this group is further augmented by the study of two new American species, for which the names *Cercaria brookoveri* and *C. macrostoma* are proposed.

Cercaria brookoveri nov. spec.

This interesting cystocercous cercaria was obtained by Professor Chas. Brookover from *Campeloma* sp. at Cedar Point, Lake Erie, April 23, 1912. The writer has been enabled to examine it thru the kindness of Professor Henry B. Ward. The worm consists of a body of typical distome characters, 0.52 to 0.62 mm. long and 0.3 to 0.37 mm. wide, a large sac-like tail trunk 1.37 mm. long and 0.46 mm. in diameter, and a pair of bluntly pointed anchor-flaps 0.45 mm. long and 0.35 mm. wide. The distome is not included within the anterior region of the tail trunk as in the previously described cercariae of this type (*C. mirabilis*, *C. wrightii*, *C. anchoroides*), but is attached at its posterior end to the trunk by a stipe, and surrounded for a short distance by a collar extension of the trunk. A cross section of the tail trunk is circular with a large central lumen, the caudal excretory trunk, while a matrix of loosely woven parenchyma cells fills the interstices between lumen and ectoderm. The excretory tube divides anterior to the anchor flaps, one branch entering each flap.

The distome is round to oval in cross section. The oral sucker, 40 by 38 μ , is directed ventrad. The acetabulum, 41 by 32 μ , is slightly anterior to the middle of the body. Above the oral sucker and slightly caudad is the pharynx, 21 μ in diameter. It leads into the intestinal ceca thru a very short esophagus. The diverticula extend laterad, then caudad, toward the posterior margin of the worm. They are somewhat convoluted and are filled with a semi-transparent jelly mass, which includes many small refractive granules and vacuoles. The

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walls of the diverticula consist of flat polygonal cells, scarcely differentiated from the body parenchyma. The excretory bladder is posterior. From it a large median tube leads forward toward the acetabulum. Its further course has not been observed. The reproductive organs are represented by a large oval cell mass posterior to the acetabulum, an elongate mass under the anterior wall of the acetabulum and a chord connecting these two masses around the right wall of the acetabulum. A comparison of size measurements of *C. brookoveri* and *C. anchoroides* seems to indicate that the two species are distinct (see table).

The nervous system is plainly detailed in frontal sections of the cercaria. There is a small cerebral ganglion mass and a subesophageal commissure almost as large. The ventrales and dorsales are easily found. Posterior to the brain the ventrales are the most conspicuous. From the subesophageal commissure two pharyngeales with intercommunicating network are derived. Along the ventral trunks the preacetabular and postacetabular commissure are prominent. There are many ventral commissures.

Like *Cercaria mirabilis* (Braun 1891) this cercaria develops within a sporocyst. The sporocyst of *C. brookoveri* reaches a length of 2.6 mm. and a cross diameter of 0.9 mm. Within it are all stages of developing cercariae from germ ball to mature larva, but only one individual of each stage is found at any one time (Figs. 2, 3, 5). The sporocyst is elongate to oval and has many prominent muscular annuli around its outer girth. As the cercaria develops the distome body is at first largest and most distinctive, with only a suggestion of the anchor flaps (Fig. 3). Later, however, the tail trunk elongates and the junction of body and tail becomes more differentiated by the formation of a stipe connection and a partial enveloping of the posterior part of the worm with the anterior part of the tail trunk. The mature larva differs from this stage in the amplification of the main trunk of the tail and the differentiation of the anchor flaps.

There is evidence to show that *C. brookoveri* is cannibalistic. Within a sporocyst of this species the larger and more mature larvae with their powerful suckers secure a hold on the less mature individuals and, with the aid of secretory juices, digest them bit by bit (Fig. 6). Thus it is probable that many germ balls are produced which never are allowed to mature because they are ingested by their more hardy congeners.

Cercaria macrostoma nov. spec.

A single specimen of this larva for which the name *Cercaria macrostoma* is proposed was found in an aquarium in the Zoological Laboratory of the University of Illinois in October, 1917. The writer

desires to thank Mr. E. C. Harrah for the specimen. The aquarium had been used for a week to contain several specimens of *Campeploma subsolidum* (Anthony) and *Goniobasis pulchella* (Anthony) before the fluke was discovered. The size of the larva and the paddle movement of the tail flaps, together with the creamy yellow color of the whole body, made the worm a conspicuous object. The movement was similar to that described by Ward (1916) for *C. anchoroides*. At first only the superficial features of the larva were evident, but under pressure of the cover slip the internal characters became clear. The trunk is a thick, oblong object, slightly conical anteriorad and wedge-shaped posteriorad. The flappers are broadly spatulate. At times they are found at right angles to the trunk, at other times they approximate one another as the wings of a butterfly. At the anterior end of the trunk there is a central median evertible proboscis surrounded by a ring of five large wartose papillae. Two of these are ventral, two lateral, and one median dorsal. Spread over the anterior two-thirds of the trunk are irregular rings of warts somewhat smaller than those around the anterior end. The larval distome is found within the anterior two-fifths of the enveloping trunk.

Cercaria macrostoma has a measurement of 5.0 mm. for the caudal trunk length and a cross diameter of 1.1 mm. Thus it exceeds *C. wrightii*, *C. anchoroides* and *C. brookoveri* in size and approximates *C. mirabilis* (Braun 1891). The flappers are 1.2 mm. long and 0.83 wide. The distome itself measures 1.2 mm. in length by 0.5 mm. in width. It is broadest near the posterior end.

The oral sucker of the larval distome is large, measuring 0.48 by 0.36 mm. in diameter, while the acetabulum is 0.20 mm. wide and 0.26 mm. long. The latter is situated somewhat posterior to the middle of the body. The oral sucker leads into a pharynx 70μ in diameter. The esophagus is very short. The ceca first run dorsad then laterad, and then continue posteriorad to the caudal region of the body. Here they curve inward and almost meet in the region ventral to the excretory bladder. They are coiled thruout their entire length. They are filled with a semitransparent yellow jelly mass in which are imbedded refractive granules. The excretory system is constructed on the plan of a long slender Y, with a slightly dilated base. The fork occurs just dorsal to the acetabulum. One pair of fine capillaries extends inward in the region of the pharynx, but the main tubule extends forward on each side around the oral sucker.

The reproductive organs are well developed, constituting a precocious condition (Fig. 7). A pair of testes and an ovary are found behind the acetabulum. In front of the acetabulum is a large cirrus pouch with thick cuticular walls and further dorsad is a large seminal

vesicle. A uterus filled with ripe eggs is found to arise in the region of the ovary, and, after coiling a short distance backward, is seen to turn to the right of the acetabulum and run to the region of the pharynx, and thence to the genital pore. The eggs measure 78 to 88 μ in length by 47 to 50 μ in cross diameter. The vitelline follicles are arranged in two loosely strung chords at the sides of the body. They extend from the region of the pharynx to the posterior region of the body. This fluke is probably an *Allocreadiine* species.

TABLE OF COMPARATIVE DATA ON CYSTOCERCOUS CERCARIAE

	<i>C. mirabilis</i> Braun	<i>C. wrightii</i> Ward	<i>C. anchoroides</i> Ward	<i>C. brookoveri</i> Faust	<i>C. macrostoma</i> Faust
Found	Free, aqua- rium	Free, aqua- rium	Free, Lake Clair	In snail	Free, aquarium
Place	Kurland	Toronto, Can.		Sandusky, O.	Urbana, Ill.
Date	1891	1885	1893	1912	1917
Larval host..	<i>Lymnaea pa- lustris cor- vus</i>	Unknown	Unknown	<i>Campelema</i> sp.	Unknown
Parthenita ...	Sporocyst	Sporocyst
Size Distome					
Length		0.45 mm.	0.64 mm.	0.52-0.62 mm.	1.2 mm.
Width		0.1 mm.	0.288 mm.	0.3-0.37 mm.	0.5 mm.
Tail					
Length	6.0 mm.	1.0 mm.	2.0 mm.	1.37 mm.	5.0 mm.
Width		0.133 mm.	0.28 mm.	0.46 mm.	1.1 mm.
Flappers					
Length	1.5 mm.	0.533 mm.	0.53-0.6 mm.	0.45 mm.	1.2 mm.
Width		0.1 mm.	0.24-0.34 mm.	0.35 mm.	0.83 mm.
Suckers					
Oral		41 μ	160 μ	40x38 μ	480x360 μ
Ventral	Larger than oral	75 μ	128-144 μ	41x32 μ	200x260 μ
Digestive Sys- tem					
Ceca			Large, heavy	Large	Crowded, espe- cially in poste- rior end
Pharynx					
Diameter			64 μ	21 μ	70 μ
Excretory Sys- tem			Reservoir reaches acetabulum	Reservoir ends little posterior to acetabulum	Reservoir con- tinues anterior to acetabulum

GENERAL CONSIDERATIONS

Aside from their anchor tail the species of this group possess other characters in common which demonstrate their close relationship. Among these are the crowded ceca with granular contents, the long median Y-shaped excretory bladder, the presence of the ovary and pair of testes close behind the acetabulum, and the swollen cirrus pouch anterior to the acetabulum. It seems significant, likewise, that Braun (1891) and Ward (1916) found wart-like protuberances on the external surfaces of the trunk, similar to those recorded for *C. macrostoma*. These species differ from *Cercaria macrocerca* de Filippi in possessing no stylet. The latter species also has no anchor flaps to the tail.

On account of the fundamental agreement of the five described species of this group, the common history of all may be learned by coordinating data from the several species. In general, the cercariae develop as the parthenogenetic offspring of sporocysts. They are found in the respiratory or digestive organs of snails. In the differentiation of the germ balls the suckers first become set off from the body, then the caudal organ is outlined. The anchor flaps first show as stubs. Somewhat later the region between body and tail becomes differentiated into a central stipe and an outer enveloping collar. In *C. brookoveri*, perhaps the least modified of all the described species, the collar is only a partial envelop. In the other four species, however, this portion of the trunk comes to surround the worm entirely save for a pore at the anterior end of the animal. Further differentiation consists in the formation of warts at various places, but most prominently around the anterior end of the worm. In *C. macrostoma* there are, in addition to the ordinary warts, five prominent papillae around the oral opening of the cyst. Ward (1916: 16) has noted that the movement of the worm is the reverse of that usually found in other groups of cercariae.

While the digestive and excretory organs are equally developed in all of the species, the genital system in *C. macrostoma* alone are sufficiently mature to warrant a suggestion of the systematic position of the group. These quite definitely relate the group to the Allocreadiidae. The limited extent of the uterus excludes these species from the Bunoderinae. The papillae on the outer surface of the worms are structurally similar to those in the Stephanophialinae, but are located on the modified portion of the tail rather than on the body of the flukes, hence are not to be considered as homologous to the oral papillae of the Stephanophialinae. The only Allocread species, the life history of which is known, is *Allocreadium isoporum* Looss. The larva of this species has a rhopalocercous structure, which is somewhat simpler than that of *C. brookoveri*. When all of the group characters are considered it seems highly probable that these five species represent a portion of the Allocreadiinae, for which there is as yet no satisfactory classification.

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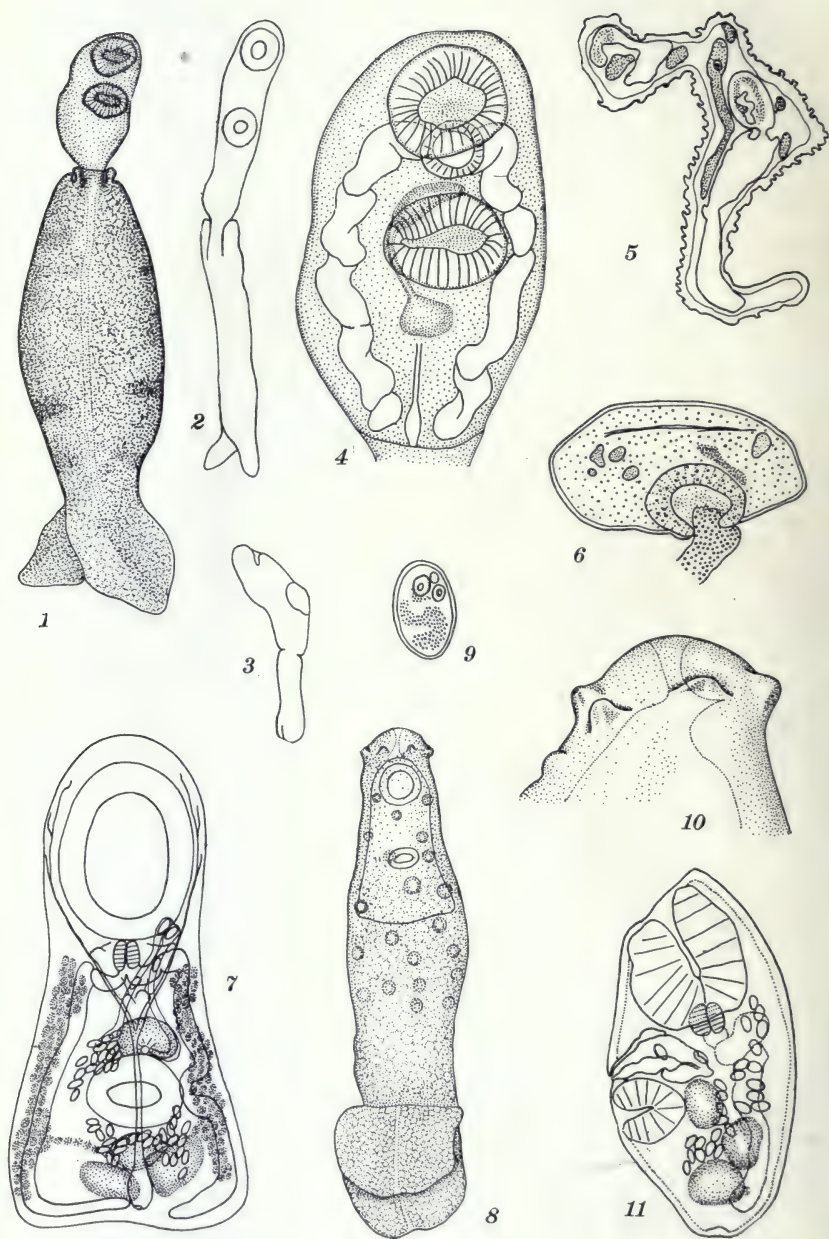


PLATE II

EXPLANATION OF PLATE

Cercaria brookoveri. 1. Sketch of entire cercaria, ventral view, $\times 34$. 2, 3. Stages in the development of the cercaria, $\times 54$. 4. Body of the worm enlarged, ventral view, $\times 105$. 5. Section of sporocyst with developing cercariae, $\times 34$. 6. Section of a maturing individual ingesting a younger individual, $\times 75$.

Cercaria macrostoma. 7. Ventral view of living fluke compressed, showing important details of digestive, excretory and genital systems, $\times 54$. 8. Sketch of entire cercaria, ventral view, $\times 14$. 9. Uterine egg, $\times 170$. 10. Detail of anterior end of cyst, showing papillae, lateral view, $\times 26$. 11. Lateral view of preserved fluke, $\times 34$.

THE TICK AS A POSSIBLE AGENT IN THE COLLOCA-
TION OF THE EGGS OF *DERMATOBIA*
HOMINIS *

L. H. DUNN

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Sufficient observations, consisting of more or less convincing facts and an abundance of circumstantial evidence relating to the egg disposal of *Dermatobia hominis*, have been reported by different writers to prove definitely that a certain species of mosquito, *Psorophora lutzii*, is the active agent in this disposal. However, neither these facts or other evidence that have been submitted up to date, are concrete enough to prove that this mosquito is the sole carrier.

In view of this doubt which still reasonably exists in regard to other insects also acting as vectors, one case of infestation with the larvae of this fly was recently brought to the attention of the writer that is considered sufficiently interesting to be worthy of report.

In February of the present year, two members of the staff of this laboratory, Dr. H. C. Clark, pathologist, and Mr. J. E. Jacob, chemist, accompanied by Mrs. Clark, left for a few weeks hunting trip near the headwaters of the Boqueron River in the interior of Panama. Aside from hunting it was also intended to make more or less of a survey of the diseases common among both the human and animal inhabitants of that region. Specimens of insects were also to be collected, especially of the groups known to be concerned in the transmission of the various insect borne diseases of man and animals.

As this was in the dry season of the year the ticks were very numerous and proved to be exceedingly troublesome pests. After being in this region about nine days, Dr. Clark, upon returning to camp after a day's hunt, on February 16, found an adult tick firmly attached to the back of his left hand. It was an unengorged female and had attached itself near the wrist joint on the median line of the hand. The locaton was at the point where a shirt or coat sleeve usually touches the hand. After the removal of the tick the site of attachment was painted with tincture of iodine followed by alcohol.

Two days later upon returning to camp, Dr. Clark found that he was again acting as a host for ticks, and that this time there were three. All three were firmly attached on portions of the body that

* Read before the Medical Association of the Isthmian Canal Zone, January 18, 1918.

were well protected with clothing. Two were located on the upper left side of the abdomen at a point above the belt line. They were attached about two inches apart. The third tick was attached on the left side of the back, also above the belt line. All three were removed and the site of each attachment well swabbed with iodine.

The four sites from which these three ticks, and the one previously found on the back of the hand, had been removed behaved like tick bites in which the proboscis had been left in the skin and delayed the healing. The tiny wounds refused to heal, although all four were treated twice a day with iodine followed by alcohol. The iodine was even introduced well down into the wound on the sharpened end of a match. Each of the four small wounds remained unchanged and continued to ooze small quantities of serum at intervals without any healing results being obtained from the iodine treatment, until February 27. On this date while returning to the Canal Zone, Dr. Clark noticed the lesion on the left hand beginning to swell like a small boil, and it soon became rather painful. This may have been hastened by using the hand rather freely in paddling a canoe. It had been suspected for several days that the presence of a larva in the wound was the cause of the non-healing. An examination was now made with the aid of a hand lens which verified the suspicion and an incision produced a *Dermatobia* larva 4 mm. in length.

Although it was now quite certain that the three lesions on the body also contained larvae, no further attention was given them except to continue the iodine treatment daily. They remained unchanged until March 6, when an area of from one-half to one and one-half inches around each wound began to swell and become red and painful. Frequent pricking and boring sensations were felt in the wounds, which at times were equivalent to the pricking of a hypodermic needle. A serous exudation was also occasionally noticed. This continued until March 8, when quite a severe glandular swelling began to take place on the left side, and it was then considered advisable to remove the larvae. Incisions were now made and a small *Dermatobia* larva removed from each wound. The smallest one was 2.5 mm. in length, and the largest one approximately 4 mm.

In order to make a positive identification of the larvae, when the first one was removed from the back of the hand it was placed in a small incision made in the skin on the back of the neck of a guinea-pig. This was done within an hour after its removal. It immediately began burrowing downward at one side of the incision. In three hours it was entirely buried under the skin, with the exception of the small posterior end, which was still visible. The following day the larva was out of sight and the incision healed over except for a small raised

area in the center. In this area a minute round crater-like opening remained. The serous fluid evacuated by the larva was being ejected through this opening. This small raised area slowly increased day by day into a swelling about three-fourths of an inch long, and over one-half inch in diameter and emitted an offensive odor. On April 7, thirty-eight days later, the guinea-pig died. Within five hours after the death of the pig the larva emerged through the small opening. It was 20 mm. in length and 6 mm. in diameter, and we were able to identify it as *D. hominis*. The larva died before pupating.

A few months later Dr. Clark became infested a second time while hunting near Arraijan, on the west side of the Canal. This infestation was also preceded by a tick attachment. On the evening of May 13, after returning from a day's hunt, a small tick evidently a nymph, was found attached on the right side of the abdomen. It was about midway between the groin and the umbilicus, and well beneath the belt line. After the removal of the tick the point of attachment was given the customary treatment of iodine and alcohol for several days without getting healing results. On May 23, a slight pricking sensation was felt in the small lesion. This pricking sensation gradually increased and the small serum-oozing crater could be plainly viewed; the larva inside was easily discernible by the use of a hand lens. The larva was allowed to remain in situ until June 4. An incision was then made and a *Dermatobia* larva, 2 mm. in length, was removed. This made five larvae of *Dermatobia* in which a tick had preceded the larva in each instance. Each larva was found in the site of a tick bite, and four of these locations were well protected by clothing.

In order that due weight may be given to the observations it must be remarked that Dr. Clark is a very close observer. He is well versed in medical entomology and is also familiar with the larvae of *Dermatobia* and the existing theories regarding the manner of disposal of the eggs.

While on the trip mosquito bars were always used while sleeping and due caution exercised while bathing, and the body protected with clothing at all times when possible. In view of these facts it would hardly have been possible for mosquitoes to reach the parts of the body where four of the larvae were situated. However, as has been shown, ticks may easily gain access to the inside of the clothing and attach themselves to almost any part of the body before being discovered.

In these cases the daily application of iodine had no appreciable effect on the young larvae. It did assist, however, in definitely marking the site of each bite, and in proving that each larva was located in a lesion primarily caused by a tick.

In the low lying territory adjacent to Gatun Lake between Gamboa and the quick waters of the Chagras ticks and mosquitoes were found equally numerous. Here the inhabitants were few and no cases of infestation with larvae of *Dermatobia* were noted among either man or animals. The river regions in the vicinity of the lower reaches of the Chagras and Pequeni rivers, between Alhajuela and Boca Culebra, were well populated and several cases of *Dermatobia* infestations were observed in domestic animals. Among the animals found to be infested, the dogs and calves seemed to be the most prolific hosts. It is needless to state that both of these animals also act as hosts for numbers of ticks. In this locality both ticks and mosquitoes were present in large numbers. As the party advanced along the Pequeni and Boqueron rivers to the higher regions the inhabitants became fewer in number. At the headwaters of the Boqueron the final camp was made, and some of the surrounding country hunted over and inspected. No inhabitants or domestic animals were found in this vicinity, but the ticks were encountered in greater abundance than in the lower regions, and were found in numbers swarming over the grass and bushes, evidently living on the wild animals such as deer, peccary, tapir, jaguar, puma, ocelot, anteater, capybara, agouti, monkey and many smaller animals with which the region abounded. In this area the higher elevation affording better drainage, the coolness of the nights, due to the high altitude, evidently proved unfavorable for mosquito breeding, as none were found at this place. No signs of *Dermatobia* larvae were observed in any of the wild animals killed in this locality, but it was at this point that Dr. Clark became infested. Lastly it is worthy of mention that of the many specimens of mosquitoes collected by the party and brought back for identification, not a single specimen of *P. lutzii* was present.

It is to be regretted that none of the five ticks that are suspected of carrying the eggs were preserved. But as these individuals were only a small part of the number that attached themselves to the different members of the party while on this trip, it is not strange that they were not preserved, as no particular attention was paid to these specimens until the lesions caused apparently by their bites failed to heal. However, other specimens found attached to different members of the party, as well as some of those that were found to be so numerous on the grass, bushes, fallen trees, etc., were brought back to the laboratory and all proved to be specimens of the Cayenne tick, *Amblyomma cajennense*. This tick has a variety of hosts, and attacks man and all classes of both domestic and wild animals with equal freedom.

In the face of this evidence it is but reasonable to consider that a species of tick, probably *A. cajennense*, not only acted as the carrier of the eggs, but was also instrumental in assisting the larvae to penetrate the skin.

The incrimination of the tick opens up a new theory on this subject and we hope it will stimulate investigations along a new line on the habits of this insect.

NEW GREGARINES FROM COLEOPTERA

MINNIE WATSON KAMM

The following pages contain descriptions of two species of gregarines which are believed to be new to literature.

GREGARINA PLATYDEMA nov. spec. (Figs. 1 to 4)

Host: *Platydema excavatum* Say (Tenebrionidae) Det. Chas. A. Hart

Location: Urbana, Illinois, June, 1917

Habitat: Intestine

The sporonts of this species are regularly biassociative, altho anomalies occur more frequently than in any other species observed by the writer. The specimens found occurred in the intestine of a tiny black tenebrionid beetle, about twelve associations and half as many cephalonts being found in each of two hosts. The maximum length of an association found was 2.41 mm.

The individual sporont is cylindrical and slender (Fig. 1), being on an average eight times as long as wide in the primate, the first member of the association, and four times as long as wide in the second member, the satellite. The protomerite of the primate is globular in shape, flattened slightly at its attachment to the deutomerite; its width and height are very nearly identical. The average ratio of LP:TL (see table at end) is about 1:12. The deutomerite is slightly constricted at its junction with the protomerite, but soon attains its maximum width which is maintained throughout the entire length, the posterior end being abruptly truncated.

The satellite differs considerably in form from the primate. The protomerite is much flattened, being only one-third to one-half as long as it is wide; it is more flattened in the large than in the small sporonts and is cupped deeply to insure a firm connection between the two members of the association. The deutomerite here is also cylindrical, bluntly rounded posteriorly. The average ratio of LP:TL is about 1:15. The width of the protomerite in the satellite bears about the same relation to the width of the deutomerite that it does in the primate: viz., 1:1.5.

Each sporont of the association is nearly transparent, no large scattered dark gray granules characterizing this species as is often true of practically transparent species.

The nucleus is conspicuous *in vivo* in both primate and satellite; it is a large sphere situated generally slightly below the center of the deutomerite and in the primate often attains a diameter of very little

* Contributions from the Zoological Laboratory of the University of Illinois, No. 117.

less than that of the sporont itself. In the satellite it is generally smaller in proportion to the diameter of the deutomerite than in the prime. One large karyosome is visible within.

Cephalonts (Fig. 3) were numerous in my material. They are stout-bodied, relatively short and broad, and the ratio of LP:TL (without the epimerite) is about 1:5, while that of WP:WD is about 1:1. The epimerite consists of a simple cone of about the same length as width surmounting the typically-shaped protomerite. This

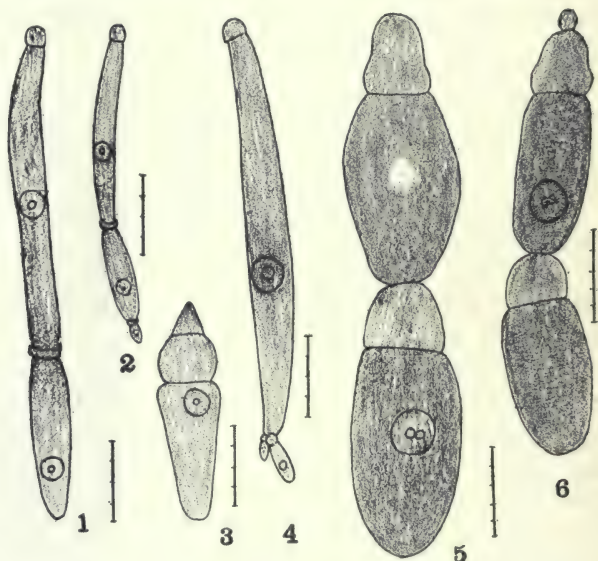


Fig. 1.—Typical association of sporonts of *Gregarina platydema*.

Fig. 2.—Atypical association, *Gregarina platydema*, consisting of a chain of three sporonts, the third minute.

Fig. 3.—Cephalont of *Gregarina platydema*.

Fig. 4.—Three atypically conjoined sporonts of *Gregarina platydema*, the satellites minute.

Fig. 5.—*Gregarina diabrotica*, characteristic association.

Fig. 6.—*Gregarina diabrotica*, abnormal group, the prime of which has not yet lost its epimerite.

In Figures 1, 2, 3 and 4 the reference line is 0.2 mm. long, and in Figures 5 and 6 it is 0.05 mm. long.

cone-shaped epimerite is unusual for the genus *gregarina* in which I place it, but similar epimerites have heretofore been reported; viz. in *Gregarina statirae* Frenzel in which it is a short cylindrical papilla rounded at the apex, and in *Gregarina acuta* (Léger) in which it is reported as a sharp point (Watson, 1916:177).

The chief peculiarity which occurs among the sporonts of this species is the great difference in the lengths of the associative sporonts, some being four times longer than others, all, however, perfectly

joined. This variation is not sudden for fairly even gradations occur, and for this reason the presence of a dimorphism cannot be entertained; the sporonts merely become associative long before they are ready for reproduction. All the free cephalonts seen were as large as many of the associative primites and when they would have attained the proportions of mature sporonts would be among the largest measured; it is, of course, true that smaller cephalonts are found embedded in the intestinal epithelium.

In one instance (Fig. 2), an association consisted of three individuals in a chain, the third being minute — one-fourth the length of the first satellite and one-eighth the length of the primite. Another irregularity (Fig. 4) consisted of a triple association, the two satellites being attached to the posterior end of the primite; both were diminutive, only about one-eighth the length of the primite.

In many species studied by the writer where hundreds of specimens were observed no abnormalities or very exceptional ones have been seen to occur. It is possible that such species as the present one are relatively new parasites to their present hosts and have not yet adjusted themselves to the conditions of parasitism offered by the hosts in question.

This species is placed in the family Gregarinidae since that family alone is characterized in part by associations of individuals with septa; and in the genus Gregarina because of the biassociation character and the shape of the epimerite. The species somewhat resembles in form *Gregarina socialis* Léger, a figure but no description or dimensions of which is given in the original reference (Léger, 1906). The latter species, however, is differentiated a), by possessing a small chromidial body in the protomerite, and b), by existing in associations of as many as ten individuals.

Dimensions in microns of several typical live specimens are given below.

	Primite				Satellite			
	a	b	c	d	a	b	c	d
Length protomerite ...	50	50	90	70	20	30	20	40
Length deutomerite...	570	800	920	1130	240	410	520	1180
Width protomerite....	60	60	90	75	50	60	70	80
Width deutomerite....	90	100	140	170	75	100	120	150
Diameter nucleus.....	35	80	70	120	35	70	60	70
Diameter nucleolus....	...	11	...	40	...	20	...	20
Total length sporont..	620	850	1010	1200	260	440	540	1210
Ratio LP : TL.....	1:12.4	1:17	1:11.2	1:17	1:13	1:15	1:27	1:30
Ratio WP : WD.....	1:1.5	1:1.6	1:1.5	1:2	1:1.5	1:1.6	1:1.7	1:1.9
Total length association	880	1290	1550	2410				

Numerous biassociative and young solitary sporonts of this species have been taken from each of a half dozen beetles of the species listed which is a pest to both wild and cultivated cucumber vines.

The sporonts are elongate-cylindrical, flattened at each end and about three times as long as the maximum width. The largest sporont seen measured 270μ by 105μ . The largest association was 530μ long. The ratio LP:TL of the primate was about 1:3.5; that of WP:WD 1:1.6.

The protomerite of the primate is broadly dome-shaped and constricted somewhat in the mid region. It is slightly longer than wide, the widest portion being in the posterior third. It again becomes constricted at the septum; the whole shape, therefore, is unique and a constant and characteristic feature of the species. The outline of the deutomerite is typical of that of many gregarines, widening immediately below the septum and retaining the same width throughout the entire length, except at the broadly rounded posterior extremity. The protomerite of the satellite is lower and lacks the constriction of that of the primate; it is regularly dome-shaped with the apex slightly flattened at its contact with the primate. The deutomerite is essentially like that of the primate.

The protoplasm is dark gray, almost black in transmitted light in the deutomerite and slightly less dense in the protomerite. The nucleus is spherical, of good size, and contains two or three minute karyosomes.

The epimerite is small, sessile and spherical, characteristic of the genus *Gregarina*, in which it is placed because of the epimerite and the biassociative sporonts. A table of a few typical measurements in microns of live sporonts follows:

	Primate				Satellite		
	a	b	c	d	a	b	c
Length protomerite	60	70	50	60	50	50	40
Length deutomerite	200	190	100	165	210	200	180
Width protomerite	60	50	40	50	70	80	50
Width deutomerite	90	90	60	70	100	105	65
Total length sporont.....	260	260	150	225	270	250	220
Ratio LP : TL.....	1:4.3	1:3.7	1:3	1:3.6	1:5.4	1:5	1:5.5
Ratio WP : WD.....	1:1.5	1:1.8	1:1.5	1:1.4	1:1.4	1:1.3	1:1.3
Total length association.....	530	510	370				
Diameter nucleus	30			

GREGARINA DIABROTICA nov. spec. (Figs. 5 and 6)

Host: *Diabrotica vittata* Fabr. (Chrysomelidae)

Location: Urbana, Illinois, June, 1917

Habitat: Intestine

This beetle is also one of the hosts of a nematode, the larvae being found in the body cavity of two specimens in countless hundreds massed tightly against the internal organs. That the larvae exert a

baneful influence upon the host is shown by the fact that after half a dozen of the host beetles had been kept in captivity for twelve hours none showed ill effects except two which were sluggish, apparently at the point of death when opened, and which proved to be heavily parasitized. The hosts must, therefore, eat regularly and often in order to feed so great a number of intruders.

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CENTRORHYNCHUS PINGUIS N. SP. FROM CHINA*

H. J. VANCLEAVE

The Acanthocephala, as a group, have a very broad geographical distribution. Practically every country, the fauna of which has been studied, has revealed considerable numbers of these worms parasitic in the various classes of its vertebrates. In going over the literature upon this group the writer has found no reference of any kind to their occurrence in China. This is probably due to the incompleteness of the published records concerning the fauna of that country and does not necessarily indicate any actual scarcity of Acanthocephala. In 1915 Dr. R. T. Shields sent material from the intestine of a magpie at Nanking, China, to Professor Henry B. Ward, who kindly turned them over to the writer for study. A study of stained whole mounts and of serial sections has demonstrated the fact that these individuals belong to a new species of the genus *Centrorhynchus* which is described below.

Centrorhynchus pinguis nov. spec.

With the characters of the genus VanCleave (1916-a). Body robust, with anterior half slightly inflated. Entire length about 15 mm.; maximum diameter in anterior third of body, about 2.5 mm.; diameter in posterior attenuated third about 1.2 mm. Proboscis about 0.77 mm. long; region anterior to insertion of proboscis receptacle ovoid, about 0.48 mm. long by 0.38 mm. in diameter; posterior to insertion of receptacle a truncated cone with the base at the line of union with body proper. Proboscis armed with about thirty-two longitudinal rows of about sixteen hooks each. Embryos 48 to 65 μ long by 24 μ in diameter; elliptical, with the three membranes concentric. Males not observed.

Host: Magpie, in intestine. Locality, Nanking, China. Type female deposited in Parasitological Collection of the University of Illinois; catalog number 18.1. Paratypes in collection of the writer.

The specimens available for this study were preserved in formalin and tho evidently killed in the same reagent, were in splendid histological condition. In toto-mounts the marked translucency of structure characteristic of formalin specimens was of great value in permitting a close examination of internal structures. However,

* Contributions from the Zoological Laboratory of the University of Illinois, No. 121.

some of the finer points of structure were determined from a series of longitudinal sections.

The proboscis receptacle (Fig. 7) is distinctly of the type characteristic for the genus *Centrorhynchus*. It is a sac shaped structure 1.3 mm. long, inserted near the middle of the proboscis and gradually diminishing in size toward its posterior extremity where it ends in a small, bluntly rounded termination. The walls of this organ are composed of two concentric layers of muscle of which the outer has a thickness of about 12μ , while the inner layer is two or three times as thick. The large invertors (*ip*) of the proboscis fill practically the entire space within the receptacle. Near the middle of the receptacle the invertors are separated by the brain (*br*), which in this species is an ovoid mass about 0.17 mm. long and 0.07 mm. broad located about 0.4 mm. anterior to the posterior tip of the receptacle. Within the brain the individual ganglion cells are ovoid in shape with a length of 41μ and a breadth of 30μ . The nuclei in these cells are very conspicuous, having a diameter of about 15μ . Fibers from the invertors of the proboscis pass through the wall of the receptacle in the region near its posterior tip and continue through the body cavity as the retractors of the proboscis receptacle (*pr*).

The body wall (Fig. 5) presents a type of structure similar to that described by the writer (1916:170) for *Arhythmorhynchus*. The high degree of development of the muscle layers (*bm*) gives the body wall in this species a peculiar appearance closely simulating that of a parenchyma. The similarity is heightened by the presence of numerous embryos (*e*) which have found their way from the body cavity proper (*bc*) into the meshwork of this loosely organized tissue. In the paper referred to above, the writer called attention to the similarity existing between the fundamental structure of the muscle cells of nematodes and of members of the genus *Arhythmorhynchus*. In *Centrorhynchus pinguis* this similarity is even more striking. Figure 6 shows the structure of a single muscle cell taken from a longitudinal section through the body wall. Each such cell is comprised of two distinct regions: a sac of undifferentiated cytoplasm (*cm*), containing the nucleus (*n*); and at the opposite end of the elongated cell a group of differentiated muscle fibrillae (*mf*). In the cells under consideration the fibrillae are restricted in their distribution to the margin of the cells contiguous to the subcuticula. In the muscle cells shown in Figure 5 these cells have been cut in an oblique plane so that only the fibrillar portion of each cell is shown. It should be kept in mind in this connection that the usual arrangement of the body muscle layer in *Acanthocephala* is such that the nuclei all lie in the dorsal region of the body. Here they occur in two more or less sharply defined

longitudinal rows. In one tangential section through a muscle cell of *C. pinguis* the writer has observed two nuclei within the same muscle cell, lying some distance apart, indicating the possibility that either the muscles of the two sides of the body are derived from large binucleate cells or are possibly the result of a fusion of the cells from the two sides of the body.

Internally, the proboscis shows a differentiation in the structure of its wall which bears a rather direct relationship to the division into anterior and posterior regions separated by the line of insertion of the proboscis receptacle. The external marking off into regions and the internal differences in structure do not, however, coincide, precisely. The wall of the anterior proboscis region is thicker and more distinctly fibrous in structure than is the wall of that part of the proboscis posterior to the insertion of the proboscis receptacle (Fig. 7). In the anterior region conspicuous groups of fibers run across the wall. The association of these fibers with the well developed root processes upon the hooks in the same region indicates a probable greater degree of freedom of movement of the hooks anterior to the insertion of the receptacle than of those in the posterior region of the proboscis.

Hooks upon the proboscis (Fig. 2) are distinctly of two types. Those anterior to the insertion of the receptacle are heavy, with conspicuous reflexed root processes, while those posterior to the insertion are more spine like and rarely possess true root processes. In these latter the basal portion of the hook or spine is embedded in the proboscis wall and alone serves for connection with that organ. The largest hooks upon the proboscis are strongly recurved, 53μ long, and with a diameter of 18μ at the point where they emerge from the proboscis wall.

The female genital tract is, in most individuals, completely obscured by the accumulation of embryos in the posterior body region. One female, stained and mounted in toto, gave a very clear view of the relations of the parts of the female genital organs as shown in Figure 4. The embryos in this species (Fig. 3) while covered with fully formed membranes display considerable degree of variability in size.

SUMMARY

The description of *Centrorhynchus pinguis*, nov. spec. from the intestine of a magpie from China furnishes apparently the first record of the occurrence of *Acanthocephala* in China. In discussing the morphology of *C. pinguis* especial attention is given to the cellular elements of the body musculature.



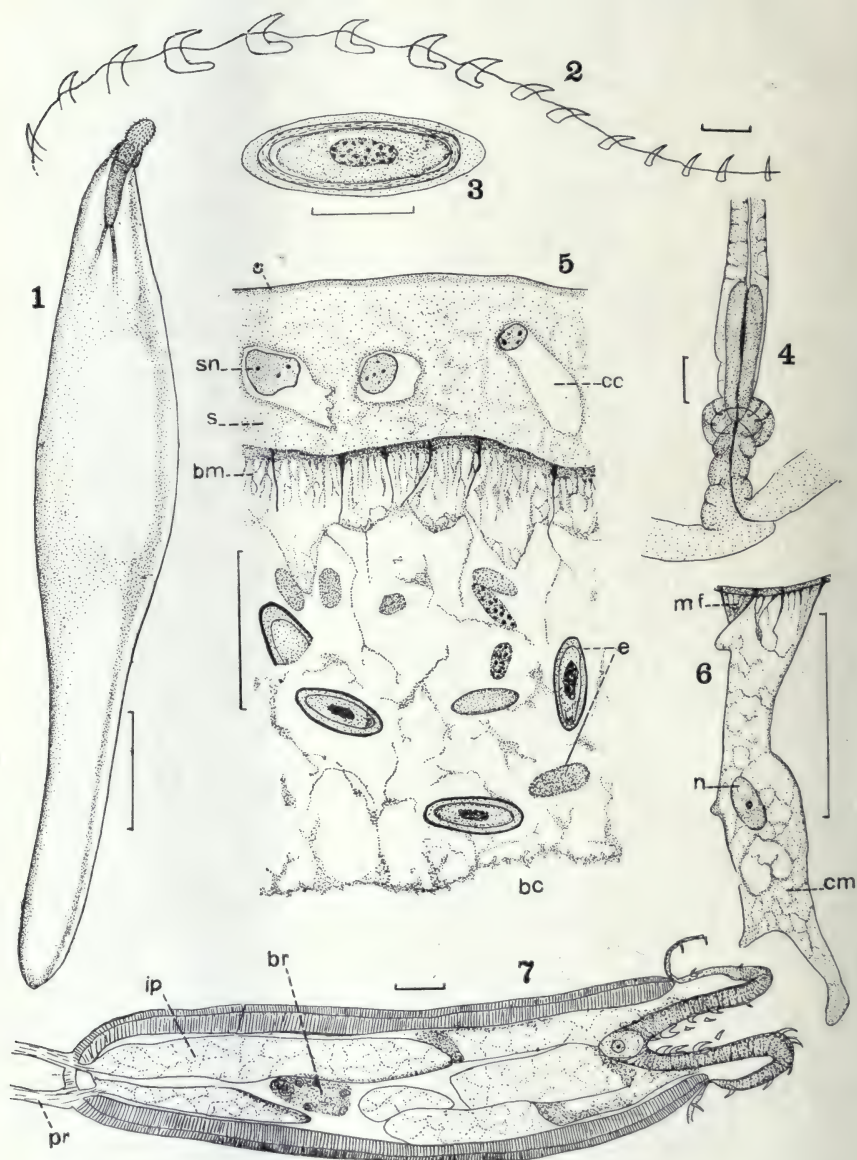


PLATE III

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EXPLANATION OF PLATE

All drawings were made with the aid of a camera lucida. The magnification of each figure is indicated by the reference line accompanying it which has the value of 0.1 mm., except that in Figure 1, it has the value of 2 mm., and in Figure 3 of 0.02 mm.

Morphology of *Centrorhynchus pinguis* nov. spec.

Fig. 1.—Type female showing general body form. From toto mount stained in Ehrlich's acid hematoxylin and mounted in damar.

Fig. 2.—Profile of proboscis of type female, showing a single longitudinal row of hooks.

Fig. 3.—Embryo from body cavity of female. Drawn from a longitudinal section of body.

Fig. 4.—Genital tract of female, from toto mount. The anterior end, including the selective apparatus, hidden from view in specimen by accumulation of embryos within body cavity.

Fig. 5.—A portion of body wall from longitudinal section. *bc*, body cavity; *c*, cuticula; *cc*, circular canal of lacunar system; *bm*, body musculature; *e*, embryo; *s*, subcuticula; *sn*, subcuticular nucleus.

Fig. 6.—A single muscle cell, drawn from longitudinal section of body wall. *cm*, undifferentiated cytoplasmic mass; *mf*, muscle fibrillae; *n*, nucleus.

Fig. 7.—Section through inverted proboscis and proboscis receptacle. *br*, brain; *ip*, inverter of proboscis; *pr*, retractor of proboscis receptacle.

A NOTE ON THE CULTIVATION OF *TRICHOMONAS* *INTESTINALIS*

MARK F. BOYD

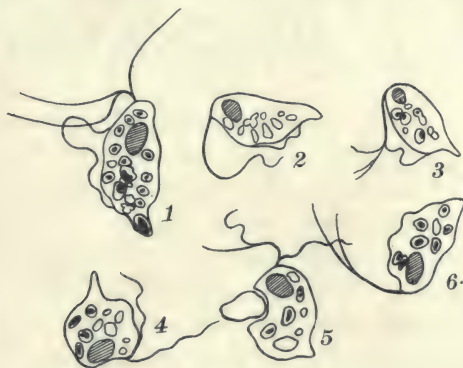
Laboratory of Bacteriology and Preventive Medicine, Medical Department,
University of Texas, Galveston

In 1915 Lynch reported the cultivation of a trichomonad secured from the gums and vagina of a negress suffering from an acute gingivitis and catarrhal vaginitis, while in 1917 Ohira and Noguchi report the cultivation of trichomonads from the dental tartar. So far as I know, no report of the cultivation of these flagellates from the intestinal tract has been made.

In making a parasitological examination of the large and small intestine of a white male removed at autopsy, the colon was found to contain a considerable quantity of very soft, light yellow fecal material. Microscopical examination of this fecal material demonstrated not over two trichomonads per slide, each slide being prepared from a single loopful of feces. Loopfuls of feces were transferred to tubes of physiological saline, acid broth (plus 1) and neutral broth, both of the latter being made from meat extract.

After three days incubation at 37° C., the supernatant fluid and sediment of bacteria and fecal debris was examined for the trichomonads. None were found in any of the inoculations made into broth, while in the sediment of the inoculations made into saline, several trichomonads were found in each field, the numbers being markedly increased over those observed in the feces from which the inoculations were made. After ten days growth, transfers of single loopfuls of sediment were made to fresh tubes of unsterilized fecal suspensions in saline, the feces employed being from a person free from this flagellate. By this time none of the broth tubes had shown a growth. After the second transfer the numbers of flagellates continued to increase, as many as a dozen being observable in a single microscopic field. After eighteen days in the second culture they were still abundant in the sediment and transfers were made to another series of unsterilized fecal suspensions. Uninoculated controls of this fecal suspension were also incubated. In the third transfer the organisms have multiplied extensively, but are apparently not as numerous as in the second transfer. The cultures are now in the fourteenth day of the third transfer, and the trichomonads are still abundant. No flagellates have been found in the control tube.

A number of the trichomonads are shown in the accompanying illustration, which was made from films fixed in sublimate alcohol and stained with Heidenhain's iron hematoxylin. Before smearing the organisms were concentrated and mixed with dilute serum water to insure fixation. Well fixed individuals show a rather elongated, pear-shaped body, blunt at the anterior extremity, pointed at the posterior. The large nucleus is visible at the anterior end, while the body cavity is filled with vacuoles containing bacteria in various stages of digestion. In well spread individuals three flagella are observable arising from the anterior end, while from the same situation arises an undulating membrane which extends towards the posterior extremity, but apparently does not reach to the tip. An axostyle has not been observed. Individuals vary in length from 12



Group of *Trichomonas intestinalis*

to 18μ and in breadth from 6 to 9μ , depending largely on the position in which fixed. These characteristics indicate the organism probably belongs to the genus *Trichomonas*, although the absence of an axostyle does not confirm this diagnosis. If the failure to observe an axostyle is due to technical errors, the species is undoubtedly *T. intestinalis*.

To date in none of the cultures have I observed cysts of any character, so that the conflicting observations of Wenyon (1910) and those of Lynch (1916) upon cyst formation in this species cannot be reconciled.

In the case from which these trichomonads were derived the autopsy revealed an aortic insufficiency, a diffuse arteriosclerosis of the kidneys, to which death was due, and a diffuse, pseudo-membranous colitis, extending from the ilio-cecal valve to the rectum.

From these observations it would appear that the recognition of intestinal flagellates might be facilitated by incubating fecal suspen-

sions in saline for several days before examining, rather than by the direct examination of fresh feces. This procedure might prove of value should it be ascertained these organisms possess pathological significance.

I shall attempt the isolation and propagation of this flagellate in pure culture with a suspension of a single bacterial species as food. It would appear that such a combination would be necessary since they appear to require the ingestion and intracellular digestion of micro-organisms for nourishment.

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ADAPTABILITY OF SCHISTOSOME LARVAE TO NEW HOSTS

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The spread of any digenetic trematode is limited by the distribution of the molluscs which serve as its intermediate host. Trematode species which can become adapted for development to several different species of intermediate hosts have a much better chance of entering new localities than those which are absolutely specific in one mollusc. The literature on the cercariae shows a number of species which can develop equally well in different species and even different genera of molluscs. The best known example of the relation of adaptability in intermediate hosts to the spreading of a digenetic trematode to new localities is the sheep liver fluke, *Fasciola hepatica*, which has become widely distributed to various parts of the world, adapting itself to some species of the snail genus *Lymnaea* found in each new region.

Although the larval stages of the human blood flukes have been known less than five years, the records already show a surprising lack of specificity in intermediate hosts. Leiper (1916:411) records the cercaria of *Schistosoma haematobium* from Egypt in *Bullinus contortus* and *Bullinus dybowskii*, and the cercaria of *Schistosoma mansoni* from *Planorbis boissyi*. Cawston (1917:133) records the cercaria of *Schistosoma haematobium* in South Africa from *Physopsis africana*, and in Venezuela Iturbe and Gonzalez (1917) found the cercaria of *Schistosoma mansoni* in *Planorbis guadelupensis* Sowerby. The cercaria of *Schistosoma japonicum* has so far been described from only one host, the katayama snail, *Blanfordia nosophora*. It is significant that this species is an operculate snail belonging to a different order of the Gastropoda from the intermediate hosts of *S. haematobium* and *S. mansoni*. In addition to these at least two of the species of forked-tailed cercaria develop as described by Faust (1915:122 and 1918:105) in more than one species of intermediate host. *Cercaria gracillima* is described from *Lymnaea proxima* Lea and *Physa gyrina* Say from the Bitter Root Valley, Montana, and *Cercaria gigas* from *Planorbis trivolvis* Say and *Physa gyrina* Say from Illinois.

My own studies on the forked-tailed cercariae from the United States have shown several striking examples of lack of specificity in the choice of intermediate host. *Cercaria douthitti* Cort which was described (1915:49) from *Lymnaea reflexa* Say taken near Chicago,

Illinois, was later found in the region of Douglas Lake, Michigan, in *Lymnaea stagnalis oppressa* (Say), *Lymnaea stagnalis perampla* Walker and *Physa ancillaria parkeri* (Cuvier). *Cercaria douglasi* Cort was found in the same region in species of snails belonging to two different genera, viz., *Physa ancillaria* Say, and *Lymnaea emarginata angulata* (Sowerby). A third species of forked-tailed cercaria, as yet undescribed, was found in a single small beach pool on the shore of Douglas Lake in species belonging to three different genera of snails, viz., *Planorbis trivolvis* Say, *Lymnaea exilis* Lea, and *Physa ancillaria* Say.

The data given above seems to clearly indicate that the forked-tailed cercariae readily adapt themselves to new molluscan intermediate hosts. Further studies on the intermediate hosts of the human schistosomes will undoubtedly add to the list of snails which can be utilized as intermediate hosts by these species. The striking dissimilarity between *Blanfordia nosophora*, the intermediate host of *Schistosoma japonicum*, and the intermediate hosts of *Schistosoma haematobium* and *S. mansoni* is also very significant in this connection. If there were any great degree of specificity in the intermediate hosts among these forms, species of the same genus would hardly be expected to develop in intermediate hosts so entirely unrelated. The close relationship of *Cercaria douthitti* and *Cercaria douglasi* to the human schistosomes, indicated in a previous publication (Cort, 1917), also makes the adaptability of these species to a variety of intermediate hosts significant in relation to specificity in the human forms.

Since the cercariae of the human schistosomes penetrate directly into their host, and can develop to maturity in rats, cats, dogs and cattle, as well as man, they will probably spread rapidly if carried into any region where suitable intermediate hosts are found. It is known from case records and records of the immigration stations that *Schistosoma japonicum* has been brought into the United States from the Orient. Before August, 1917, when schistosomiasis was placed on the exclusion list by the Surgeon-General of the United States Public Health Service, orientals with this disease are known to have entered this country in considerable numbers. In many of the irrigated regions of the Pacific Coast states, oriental laborers from countries in which schistosomiasis is prevalent live in much the same relation to the soil as in their own country, making ideal conditions for the spread of this disease provided a type of snail in which the flukes can develop is present. It is, therefore, evident that the question of the adaptability of the schistosomes to new intermediate hosts becomes a problem of great significance in relation to the possible

spread of this disease in the United States, and it is of great importance to discover whether there are snails in this country in which the blood flukes can develop.

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ON THE PARASITISM OF CARBONIFEROUS CRINOIDS *

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The nature of the evidences of parasitism in geological epochs will doubtless be of interest to all parasitologists. No statement concerning parasitic conditions among fossil animals is made in any of the usual text-books of paleontology, and the general reference works of zoology make no mention of the matter. Abel (1912), however, in his excellent work devotes two paragraphs to a review of possible conditions of parasitism among fossil animals, calling attention especially to the work of von Graff on the swollen stems of Carboniferous crinoids of Germany. Stromer von Reichenbach (1909) refers to parasitism among fossil corals, and figures a cross section of *Pleurodictyum problematicum* from the Lower Devonian of Eifel. This is regarded by Abel as an example of symbiosis. It seems quite probable that Abel is correct in his interpretation.

Robert Etheridge (1880) was the student who first recognized the nature of the swollen stems of fossil crinoids, though he was unable to determine the nature of the parasite. This was later accomplished by L. von Graff (1885), who was able to determine the nature of the parasite, having discovered the carbonized remains of one of the myzostomids which he regarded as the infecting form. Graff reviewed the literature and referred to numerous species of crinoids which showed swollen stems, some of the species having been based on these swollen stems, which were mistaken for calyces. Graff compared very carefully his results with the swollen crinoid stems as described for recent forms in the Challenger reports, where the infecting forms were known to be myzostomids.

John M. Clarke (1908) has written an excellent paper on the pre-carboniferous evidences of communism and commensalism, calling his study "The Beginnings of Dependent Life." In his extensive collections he has found no trace of definite parasitism, but certainly the cases described by him may be regarded as the beginnings of parasitism. It seems probable at present that true parasitism did not begin until the Carboniferous Period.

Swollen stems of crinoids have often been seen by paleontologists both in America and in Europe, but few have recognized their para-

* An abstract of this paper was published in the Proceedings of the American Society of Zoologists, Dec. 27, 1917, p. 34.

sitic nature. A few species and genera of fossil crinoids have been based on the enlarged stems, the specimens being regarded as aberrant calyces.

The specimens of crinoid stems at the writer's disposal are the first to be recognized in America as many ways suggesting parasitism. Specimens are fairly common in collections of fossil invertebrates, and especially so from the Keokuk beds, where the swollen stems often assume a geoditic nature, which, owing to complete mineralization, destroys the anatomical details and leaves only the outward form.

There is nothing to be added to what is already known concerning the parasitism of Carboniferous crinoids, save that this is the first record made of swollen crinoid stems in America. They have frequently been seen, but so far as I can determine, their nature has never been recognized. There is so little difference between the American specimens and those described by von Graff and Etheridge that a very brief description will suffice.

The specimens vary from a half inch to four inches in maximum diameter, the plates of the stems being enlarged and spread apart. The columnars are often spread out to four or five times their normal diameter, the space between the series of columnars being widened to several millimeters. The individual plates are not separated. The enlargements are often mere bulgings in the stem, and again they take the appearance of large tumors, tapering at each end to join the stem. It is impossible in the present case to determine the location of the parasite, but Graff found the parasite located near the point of greatest enlargement of the stem. The swelling of the stem usually does not distort its pentagonal symmetry.

It should be noted here that Bassler (1908) has described objects of a similar nature, and has interpreted them as due to the geodization of the fragment of stem. The objects I have studied are, however, entirely different from the specimens studied by Bassler, judging from his figures and descriptions. There can be no doubt that many enlarged crinoid stems do not represent parasitism, but are the result of the formation of the geode. Many of them, however, may represent parasitism, and paleontologists have not, to date, taken this fact seriously into consideration.

The writer's interest in these objects is due to the fact that the swollen stems must be regarded as the first evidences of disease in geological history. So far as known no fossil animals suffered from disease prior to the Carboniferous, and these tumor-like masses in the stems of crinoids must be regarded as the earliest evidences of pathological processes. Diseased conditions became more and more apparent from the Carboniferous Period down to the present and disease is more prevalent today than ever before in the history of the world.

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 REVIEW AND NOTES

INFECTION AND RESISTANCE. Hans Zinnser, M.D. With a chapter on Colloids and Colloidal Reactions by Prof. Stewart W. Young. Second edition, revised. The Macmillan Company, 1918. xiii + 585 pages. \$4.25.

The second edition of this admirable and scholarly work was prepared, as the author says, under difficult conditions—far from the facilities of libraries and files of reprints. Nevertheless, the changes represent fairly the advances in the field of knowledge since the first edition was completed.

To the chapter on anaphylaxis has been added a wealth of new material. The section on infection and immunity in poliomyelitis is entirely new, as also an extended discussion of immunity in syphilis. A whole chapter has been added on serum enzymes, leukocytic enzymes, on the physical factors which enter into serum reactions, and on colloidal gold reaction. The book has appealed, and will continue to appeal, to those who are looking for insight into the fundamental principles on which rests our knowledge of infectious diseases. Every student of medicine should know and use this work in preparation for the handling of cases in clinic or laboratory.

 NOTES

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STUDIES ON THE IGUANA TICK, *AMBLYOMMA* *DISSIMILE*, IN PANAMA *

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Some months ago while engaged in making a collection of the various species of snakes commonly found on the Isthmus of Panama, my attention was attracted by the number of specimens that were acting as hosts for the "iguana tick," *Amblyomma dissimile*. Over 60 per cent. of the total number of specimens collected were found to have ticks of this species attached to them. The 40 per cent. found to be free from ticks included a few that were aquatic in habits and a number of terrestrial varieties that were of a burrowing nature. Naturally, with such habits tick infestation is highly improbable. If only those specimens with habits *compatible* with infestation, such as the non-burrowing terrestrial and arboreal varieties, are taken, the numbers found with their parasitic associates tightly attached to them greatly exceed 60 per cent.

Besides being attached to snakes, this tick is also commonly found on toads and iguanas. Out of a number of these two latter animals received at this laboratory during the past two years, about 72 per cent. of the toads and 84 per cent. of the iguanas were found to be infested. Possibly this species may also attach itself to tortoises, small lizards, and other cold blooded animals, but so far the snakes, iguanas and toads have been found to be the common or representative hosts on the Isthmus.

On the snakes that have been collected I have found specimens of this tick in different stages of development, from the larval form to the sexually mature males and replete females. The larvae were found to be the more numerous. With some of the larger snakes a close search was necessary to find the small unengorged larvae, as they usually attach themselves beneath the scales of the host and are, partly or entirely concealed, but a scrutiny beneath the scales usually resulted in exposing larvae. A slight raising of the scales of reptiles is strongly indicative of either the presence of larval ticks or small

* Read before the Medical Association of the Isthmian Canal Zone.

swellings caused by their having been attached previously. The nymphs when unengorged were also frequently found partly concealed beneath large scales, but when engorged were easily noticeable. Unengorged males and females were usually quite prominent. Altho the engorged females were not as numerous as the other forms, quite a number of them were found and in some instances were hanging from the side of the snake like globular pendants.

As the female of *A. dissimile* when fully replete is considerably larger than the other species of ticks found on the Isthmus — with possibly two exceptions — one may suspect that many of them before becoming fully engorged, are detached by the host passing swiftly thru thick grass, bushes and trees, over rough ground, sharp stones, etc. The engorged pendant females are prominent enough to catch on the many sharp edges of these objects and it is quite possible that many of them are torn loose in this manner during the travels of the host. It was noted that the ticks on the dorsal engorge to a larger size than those on the lateral or ventral surfaces. This is probably due to the fact that they escape the friction that those on the lower surfaces are subjected to, and also that those on the dorsum may use their claws and pulvilli to assist in holding them in position, while those on the sides and venter cling almost solely by their mouth parts.

Although *A. dissimile* has no apparent economic importance, a few studies were made on the bionomics and life history of this species in Panama which may be worthy of recording for the purpose of comparison with observations made on this tick in other localities. At the beginning of my observations, considerable difficulty was experienced in keeping suitable cold blooded hosts alive. Experiments were first tried with large toads, but they usually died within a few days when confined in cages small enough to control the dropping of the ticks. When placed in large cages and supplied with proper food they lived for considerable periods of time, but in the larger cages the smaller forms of the ticks could not be effectually controlled. As this did not give a definite record of the different stages, the use of toads for hosts was abandoned after a few trials. Iguanas were next tried and rejected for the same reason. Snakes gave the most satisfactory results as hosts.

Glass jars of three gallons capacity were used for containers. The first method adopted consisted in placing a snake in a jar and then dropping in the ticks to be observed. The top of the jar was then covered with a piece of light muslin, held in place with wide rubber bands. This prevented the escape of any of the ticks, yet allowed a sufficient air supply to prevent asphyxiating the host. Two faults were apparent with this method. First, that it necessitated the removal of the host from the container twice each day in order to search for

ticks that had dropped, many of them being hidden between the coils of the snake and could not be found without removing the latter from the jar. Second, the snake lying on the bottom of the jar created a certain amount of moisture which had a tendency to affect the ticks.

In order to correct these faults it was necessary to devise an arrangement to keep the snake from resting on the bottom of the jar. This consisted in cutting out a circular piece of wire screening about one-half inch larger in diameter than the inside of the jar. This extra one-half inch was then turned down at a right angle all around the edge. A disk of white blotting paper was cut out and placed in the bottom of the jar, the screen with the turned edge downward then being placed on this paper disk. The turned edge of the screen kept it one-half inch above the paper. The snake was then placed in the jar on the screen, the mesh of which being one-half inch, allowed the ticks to drop thru on the paper as soon as they detached from the host. Usually they crawled beneath the paper on the bottom and could easily be noticed there by elevating the jar. The screen fitting the bottom of the jar tightly could not be displaced by the movements of the snake, which latter were always too large to crawl through the meshes. This arrangement proved to be very satisfactory.

The serpents used for hosts were adolescent specimens of the large boa, *Boa imperator*, mature specimens of the rainbow boa, *Epicrates cenchria*, and the tree snake *Oxybelis fulgidus*. The specimens selected were as large as the size of the jars would comfortably accommodate.

A series of four rearing experiments were conducted, of which I record but one. This one experiment not only represents a complete life cycle of *A. dissimile* for this region but also illustrates an instance of part of the larvae dropping to molt while the remainder molted on the host. This occurred in but one out of four experiments.

On September 17, 1916, a live specimen of *Epicrates cenchria* was captured near Balboa and presented to the laboratory. When received a female *A. dissimile* was found attached on the dorsal surface about 8 inches from the snake's head. Although but about three-fourths engorged, this tick was quite large. Engorgement was completed four days later and the tick dropped from the host on September 21. This replete female which was the largest specimen of *A. dissimile* that I have observed, measured 25 mm. in length, 15.5 mm. in width, 9 mm. in height and weighed 2.4 grams. After a preoviposition period of six days this female began depositing her eggs on September 27. Daily oviposition continued until October 31; covering a period of thirty-five days. The total number of eggs deposited was 9,254, which is the maximum observed from this species.

The eggs of this species are very light brown in color when first deposited and thinly coated with a transparent viscid substance which

dries very quickly. A slight greenish tinge becomes apparent in a few days which gradually disappears as the eggs assume a darker shade of brown. Twenty-two days after they were deposited a white spot appeared on the side of each egg, caused by the development of the young embryo within the egg. These white spots becoming more pronounced as the embryos continued to develop, the eggs soon became considerably lighter on one side than on the other. An average egg measured 0.7 mm. in length, 0.5 mm. in diameter and weighed 0.143 milligrams.

The first eggs of this lot began hatching on November 6, and the last ones on December 8. This gave an average incubation period for each day's oviposition of practically 40 days. They all required about the same period of incubation.

When the young larvae emerged, the white spots noticed through the walls of the eggs were very pronounced and became more so when the larvae engorged. A newly emerged larva of average size measured 0.9 mm. in length, 0.5 mm. in width, weighed 0.2 mgm. and varied from gray to brown in color.

Within forty-eight hours after emerging the larvae separated from the mass of empty egg shells and swarmed in a cluster on the under side of the cover of the petri dish in which they were confined.

On December 30 about 200 of these young larvae were sprinkled over a constricting snake, *Boa imperator*. Within a few minutes they were actively crawling over the snake, and it appeared that when any of the scales were raised by its turning the larvae were ready to make their way beneath them. Many of them attached within fifteen minutes. When ready to attach the larva spread its legs, and after securing a grip on the skin with its claws, proceeded to force the hypostome into the skin, the palps meanwhile separating and spreading outward. It seemed to require but a few minutes and slight effort to insert the hypostome to full length. When this was accomplished the palps were extended at nearly right angles from it.

On the second day all unattached larvae were removed from the jar. The greater number of those attached were located on the dorsal surface, many of them being on the median line; some were also scattered over the lateral surfaces and a few were found on the ventral surface close to the vent.

As these larvae all began to engorge, the many scales beneath which they were attached became slightly elevated and caused the snake to have a peculiar rough appearance in place of the smoothness usually present.

Ninety-four engorged larvae dropped between January 10 and January 17 after an attachment period of from 11 to 18 days, the

greater number detaching on the thirteenth day. The remaining larvae, altho all appeared to be completely engorged, maintained their attachment at the host and did not drop.

A replete larva of average size measured 2.2 mm. in length, 1.7 mm. in width, 0.7 mm. in height, and weighed 1.5 mgm. The color varied from pale drab to light brown and chestnut brown.

At from 6 to 10 days after dropping, during which time they moved about but very little and that rather slowly, the first stages of molting was manifested and they became practically motionless. At this time while the change from larva to nymph was slowly taking place, the outer skin became dry and shriveled with a white transparent appearance at the anterior end, which appearance increased as the changes within continued.

The nymphs all emerged between January 24 and January 28, the molting period being from 11 to 15 days, with the greater number emerging on the fourteenth day. However, in our other rearing experiments we have found larvae that engorged on toads to require as long as 19 days to molt. A few hours after emerging the young nymphs became quite active and traveled rapidly about in the rearing jars in which they were confined. At this stage their legs appeared to be out of proportion in length to their small bodies.

An unengorged nymph measured 2 mm. in length, 1.5 mm. in width, 0.7 mm. in height and weighed 0.8 mgm.

On February 15 these nymphs were placed on a "tree snake," *Oxybelis fulgidus*, which was 4 feet in length and about three quarters of an inch at its largest diameter. Many of the larvae attached quite readily, those that did not attach within 24 hours being removed from the jar. The first one to leave the host dropped on February 27, requiring but 11 days to engorge. The last one dropped on March 9 after a 22 day engorgement period. Among those that dropped last were a few that appeared to have been completely engorged for several days before dropping.

An average engorged nymph measured 5 mm. in length, 3.5 mm. in width, 2 mm. in height, weighed 29.5 mgm. As with the larvae, the color was not constant and varied with individuals, from a light drab to a deep brown. After a molting period of from 8 to 16 days, with the greater number emerging on the fourteenth day, the adults began to emerge. The first one emerged on March 14 and the last one on March 23. As the individuals that remained attached to the host the longest were those having the shortest molting period—the nymph with the 22 day attachment period molted in 8 days—it is possible that they remained attached for several days after apparent repletion and the molting process had begun before dropping occurred.

A newly emerged adult female measured 5.5 mm. in length, 4 mm. in width and weighed 7 mgm. The coloration was reddish brown with lighter colored ornamentations on the scutum.

It will now be necessary to return to the engorged larvae that were left on the *Boa imperator*. As previously stated, ninety-four of the larvae dropped engorged between January 10 and January 17 while the remainder did not detach altho apparently engorged. These larvae remained on the host and molted, and the nymphs also became engorged before dropping. The replete nymphs began dropping on February 8. The last one detached on February 28. After all the nymphs of this lot had dropped, the snake was examined closely and a few of the small cast larval skins were found still attached beneath the scales. During our many rearing experiments with this tick, this is the only instance in which molting occurred on the host, and we are unable to explain what caused the irregularity in this case. However, we are glad to have been able to witness this departure from the usual procedure, as a considerable difference of opinion seems to exist regarding the life history of *A. dissimile*. Newstead (1909), in writing of this species, states: "Both the nymphs and the females mature very slowly, and it is evident that all three stages (larva, nymph and adult) are passed upon one host; so that in this respect it differs markedly from its congeneric representative, *Amblyomma cajennense*, which requires three hosts and effects its two molts upon the ground. The life cycle of *A. dissimile*, therefore, resembles that of the common cattle tick (*Margaropus annulatus australis*). Hooker, Bishopp and Wood (1912), who found that this species dropped to molt and required three hosts, in mentioning the observations by Newstead say, "The only information upon the biology of this tick that the authors have found is furnished by Newstead (1909). This author is in error in supposing that the molts are passed upon the host, as such is not the case."

After observing the instance of part of the larvae dropping to molt while the remainder molted on the host, as previously stated, we can more easily understand the difference of opinion regarding the biology of this tick.

Thirty-four adults, 17 males and 17 females, were placed on a "rainbow boa" *Epicrates cenchria*, about 4½ feet in length, on April 9. Although these adults had remained quite dormant after emerging, they became very active when placed on a host and several were attached within twenty minutes. The following morning, April 10, four were found to be still unattached and were removed from the jar. The thirty that were attached, 16 females and 14 males, were all located on the dorsal surface of the host. Eleven of these were grouped

in a small area, less than $\frac{3}{4}$ of an inch in diameter, about 4 inches from the head of the snake. Three inches from this group another cluster of eight were attached so closely together that they were lying partly on top of each other. The rest were scattered about over the dorsal and lateral surfaces. On April 11 it was noticed that some changing about had occurred and the number of individuals in the first cluster had increased to nineteen males and females intermingled. Several days passed without any signs of engorgement taking place, during which time the males detached and moved about quite frequently, and it is probable that copulation, which evidently takes place on the host, was necessary before the females began to engorge to any extent.

As the females became nearly engorged they began excreting small drops of white, chalky-like fluid. The first replete female left the host on April 26, after a 17-day attachment period, and nine more dropped between this date and April 30. An ulcer which had been developing at the area where the large cluster of ticks were attached became so serious at this time that the rest of the ticks were removed in order to save the snake. The lot removed were found in all stages of engorgement, from those fully engorged and about ready to detach to several that evinced no signs of repletion except for a slight thickening.

The engorgement period of the adult female seems to be quite variable; during the whole of our rearing experiments with this tick we have found the shortest period to be 15 days and the longest 36 days.

The average adult male when taken from a host was 5 mm. long, 3 mm. wide and was quite flat. It weighed 10.8 mgm., and the predominating color was brown with light ornamentations.

The coloration of an engorged female varied from gray to dark brown with yellowish markings; the scutum which was usually dark brown also having markings of a lighter hue. The integument of the entire body was thickly dotted with small, round, black spots, with a minute integumental pore in the center of each spot.

Altho these ten females were all apparently fully replete the weights and measurements varied considerably as shown by the following table:

TABLE 1.—WEIGHTS AND MEASUREMENTS OF ENGORGED FEMALES

Tick	Weight, Gm.	Length, Mm.	Width, Mm.	Height, Mm.
1	1.6845	19	14	9
2	1.5259	20	13	8
3	0.7175	16	10	7
4	1.6957	21	14	10
5	0.6768	15	9	6
6	1.2876	19	13	8
7	1.4980	21	13	8
8	0.9108	18	12	7
9	1.4776	20	13	8
10	1.6892	22	14	8

These ten females were placed in separate petri-dishes consecutively numbered and kept on a slightly darkened shelf out of all direct sunlight. During the first 2 or 3 days after dropping from the host, small quantities of the same chalk like fluid, discharged during the few days prior to dropping, was excreted by each female. Beginning within a few hours after dropping, and with some of the individuals extending up until several days after oviposition began, a small globule of serous like fluid was noticed issuing from each of the tiny pores located within the small black spots with which the integument was thickly dotted.

The period of preoviposition was quite short; the number of days elapsing between the dropping of the ticks to the beginning of oviposition is given below:

TABLE 2.—PREOVIPOSITION PERIODS

Tick No.	1	2	3	4	5	6	7	8	9	10
Days	5	5	5	7	6	4	4	4	5	4

When oviposition began the eggs were counted each morning, and those deposited during the preceding twenty-four hours were removed to a separate petri-dish. As oviposition continued and the females became depleted, the yellowish brown patterns on the dorsal surfaces became lighter in color and more pronounced.

The following table shows the oviposition periods and the number of eggs deposited by each tick daily:

TABLE 3.—DAILY OVIPOSITION

Date, May	Number of Eggs Deposited by Each Tick									
	1	2	3	4	5	6	7	8	9	10
1	406									
2	832	291	501			209	464			
3	468	720	640			631	733	533		
4	801	681	544		291	670	777	503		32
5	614	746	401	187	294	516	725	574	384	404
6	473	556	314	396	356	563	652	542		504
7		546	410	582	314	651	633	568		859
8		520	261	612	315	529	643	486		682
9		586	215	906	230	420	595	404	509	510
10		509	170	582	193	401	551	381	744	491
11		425	116	645	139	311	419	278	884	311
12		424	69	721	48	290	359	130	642	399
13		400	46	502	9	236	194	107	745	481
14		329	31	402		143	243	61	694	523
15		231	20	384		137	208	53	388	539
16		205	12	256		73	98	32	343	329
17		155	6	273		41	86	28	264	275
18		135	2	285		22	71	14	200	181
19		88	7	101		5	42	11	113	248
20		48		73			34	6	71	189
21		46		4			20	8	64	53
22		47					12	2	9	
23		21					7			
24		14		10			3			
25		6								
26		5		7						
Totals	3,594	7,684	3,765	6,878	2,189	5,848	7,564	4,673	6,054	7,010

The shortest incubation period of these eggs was thirty-eight days, and the longest forty-seven days.

Thruout this series of rearing experiments all the ticks were kept in the shade between two open windows. The temperature thruout the period during which these observations were conducted is given below.

TABLE 4.—AVERAGE TEMPERATURES PER MONTH

Months	Average Maximum	Average Minimum	Average Mean
	°F	°F	°F
September.....	85.4	73.3	79.4
October.....	82.8	73.2	78
November.....	83.8	72.3	78.1
December.....	86.2	72	79.1
January.....	86.6	70	78.3
February.....	86.9	69.4	78
March.....	87.8	72.1	80
April.....	88.4	72.2	80.3
May.....	85.2	73.4	79.3

No experiments were made to determine whether or not this tick in its various stages would attach to warm blooded hosts except in one instance when twenty larvae were placed in an uncovered pill box applied to the arm of the writer. This box was held with elastic bands for over 5 hours, but none of the larve attached during that time.

A few tests were made to determine the longevity of *A. dissimile* in the different stages of development. Of a number of larvae that were placed in a test tube on November 18, 1916, and allowed to remain perfectly dry about 5 per cent. remained alive for 101 days without any moisture. When confined in a large stender dish containing sand which was occasionally moistened some of the larvae lived for a period of 228 days. Nymphs when placed in a fairly damp situation remained alive for 162 days, but it was noticed that when placed in surroundings damp enough to cause the growth of molds that the nymphs died in less than 10 days. The greatest longevity period for unengorged females in the presence of moisture was 147 days.

No detailed observations were made to determine the length of time that adult males remained on a host, but at the present time a *Boa imperator* at the laboratory has two adult males attached to it that have been in situ for over 7 months. They have remained attached at apparently the same place during the whole of that time, regardless of the host shedding its skin several times during their attachment. This seems to be contrary to their habits when females are also present on a host.

In order to determine the length of submersion compatible with the life of this species a number of unengorged females were placed in a jar of water. They soon sank to the bottom and in about two hours became motionless. A few were removed each day to see if they were alive. When first taken from the water they always remained without motion and appeared to be dead for several hours, but if placed in the sunlight or other warm situation they became very

active at the end of this time. The last female was taken from the water on the seventh day of submersion and was found to be alive.

When a large number of adults are present the habit of attaching in clusters often causes the death of the host when the latter happens to be a snake. We have had several specimens of serpents die from the effects of tick infestation when an excessive number was applied.

Usually the snake did not seem to mind when the ticks attached themselves except in extreme cases. We observed one highly nervous snake become very angry and excited when a female persisted in attempting to attach at the inner edge of the snake's upper lip. The latter shook its head violently and struck repeatedly at the side of the glass jar in an attempt to dislodge its tormentor. No other indications were noticed to show that reptiles and batrachians ever made any attempt to remove the ticks from themselves with their mouths or bite them.

If the skin of a snake, on which a number of ticks have been attached, is examined closely prior to shedding, it will be found to be very rough and covered with small abrasions and dried scabs.

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CONTRIBUTIONS TO THE STUDY OF PARASITIC PROTOZOA. IV.

NOTE ON SOME MYXOSPORIDIA FROM CERTAIN FISH IN THE VICINITY
OF WOODS HOLE *

ROKUSABURO KUDO

The following is a brief summary of observations made on Cnidosporidian parasites of certain fish in the vicinity of Woods Hole in the summer of 1916. Six species of the parasites were found in eighteen species of fish, chiefly of salt water, which came under my observation. The names of the fish, together with those of the parasites, are shown in the following table.

TABLE 1

Species	Number of Fish Examined	Number of Fish Having Parasites	Organs Harboring Parasites	Parasites
<i>Anguilla chrysopa</i>	3			
<i>Bothus maculatus</i>	1			
<i>Centropristes striatus</i>	2			
<i>Esox</i> . sp.	3			
<i>Fundulus heteroclitis</i>	78	34	Gill	<i>Myxosoma funduli</i> n. sp.
<i>Fundulus majalis</i>				
<i>Menidia</i> sp.	3			
<i>Morone americana</i>	12			
<i>Mustelus canis</i>	1			
<i>Paralichthys dentatus</i>	15	7	Gallbladder	<i>Ceratomyxa drepanosettae</i> Awerinzew
<i>Perca flavescens</i>	30	1	Spleen	<i>Myxobolus pyriformis</i> Thélohan
<i>Phycis tenuis</i>	1	1	Gallbladder	Small number of Myxosporidia in vegetative stage
<i>Prionolus carolinus</i>	12			
<i>Raja erinacea</i>	1	1	Gallbladder	Small number of Myxosporidia in vegetative stage
<i>Rhombus triacanthus</i>	11			
<i>Scomber scombrus</i> *.....	20			
<i>Stenotomus chrysops</i>	14	4	Gallbladder	<i>Ceratomyxa</i> sp.
<i>Tautaga omitis</i>	5			
<i>Tautoglabrus adspersus</i>	10			

* It is quite remarkable to note that Fantham and Porter (1912) found 4 out of 25 fish from French waters which harbored a Myxosporidium.

* From the Marine Biological Laboratory, Woods Hole, and the Laboratories of the Rockefeller Institute for Medical Research.

The fish were brought into the laboratory alive. A study of the external characters and the branchial lamellae under a dissecting microscope was followed by a microscopical examination of the liver, spleen, kidney, urinary bladder and gallbladder. The dark field microscope was used to a greater extent than the ordinary microscope.

For the extrusion of the polar filament, I have been using in my recent work chiefly mechanical pressure and alkali. Perhydrol, which was proved to be an energetic reagent for *Nosema bombycis* (1918) was used on the spores of various Cnidosporidia with satisfactory results. Fixing and staining were usually done as described in previous papers, with a few modifications.

Myxosoma funduli nov. spec.

Habitat: The branchial lamellae and connective tissue of the gill-filament of *Fundulus heteroclitis* and *F. majalis*.

The cysts distinguish themselves from the gill as small white spots attached to the surface of the gill (Fig. 2). In the case of heavy infection, it is not uncommon to find many cysts in one line parallel to the free end of the gill (Fig. 1), similar to those observed by Müller (1841, Fig. 7).

The form of the cysts is usually spherical (Fig. 2), the average diameter being about 150μ . The largest one, however, which was encountered, measured 360μ by 264μ . All the cysts found in sections were in the final stage of development, so that young and matured spores only could be observed.

The spore is pyriform (Text figure A, Figs. 3-7). The spore coat, which is usually uniform in thickness, shows seven to ten markings on the posterior half of its surface (Text fig. A, *a*, *d*, and Fig. 3). A process similar to that of *Myxosoma dujardini* and *Myxobolus toyamai* was found occasionally (Text fig. A, *c*). The spore-membrane is composed of two halves, the line of junction being parallel to the line connecting the two polar capsules (Text fig. A, *b*). It is straight and thickened a little along the line of junction. The average dimensions of the spores are 14μ in length, 8μ in breadth, and 6μ in thickness. The optical cross-section, therefore, is always oval (Fig. 4).

Two polar capsules and the sporoplasm are found in the sporecoat. The former is pyriform in shape, with dimensions of 8μ in length and 2μ in width. The length of the polar filament is 38 to 42μ . Figure 6 shows one of the filaments extruded under mechanical pressure, though not at its full length. Pressure also changes the form of the spore. For this reason, perhydrol is much to be preferred to pressure, as may be seen from a comparison of figures 5 and 7.

The sporoplasm is granular in structure. Two nuclei were detected in stained preparations (Text fig. A, *d*). In fresh preparations the

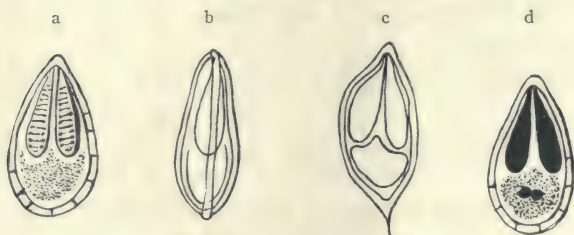
faint outline of a vacuole-like structure was occasionally observed in the sporoplasm. No iodophile vacuole, however, appears when the spore is treated with iodine water, iodine alcohol, or Lugol's solution.

Hahn (1913, 1917) described *Myxobolus musculi* found in the integument, muscles, and gills of fish of the same species in the same locality. During my examination I failed to encounter any parasites in the integument.

The spore of *Myxobolus musculi*, according to Hahn, is 14.3μ long and 6μ wide and has two polar capsules (2 by 0.5μ). Moreover, it has an iodophile vacuole in the sporoplasm and has no marking on the coat. He proved by an infection experiment that the *Myxobolus* found in the gill and that found in the muscle of *Fundulus* were the same, though he seems to have noticed some different characters, as for example, a difference in the form of the parasite.

Linton described a Myxosporidian found in the integument of *Cyprinodon variegatus* from the same locality, which was also observed and described by Gurley as *Myxobolus lintoni* Gurley. The form of

TEXT FIG. A



the spore of the species mentioned is somewhat similar to the present form, the latter, however, being narrower at the anterior half than the former. The dimensions given by Linton and Gurley are: length 13.9μ , breadth 11μ and thickness 8μ . Gurley detected an iodophile vacuole in the sporoplasm; this was not the case with the form in question.

The absolute absence of any iodophile vacuole in the present species leads me to describe it as belonging to the genus *Myxosoma* (Thélohan), which Gurley included in the genus *Chloromyxum*.

Of the two species hitherto known, *Myxosoma ambiguum* Thélohan is quite different from the one in question. *Myxosoma dujardini* has a spore similar in size and form to the present form but without the marking on the coat. Moreover, the spore-coat of the species above-mentioned is typically more thickened at the anterior end than in the one I observed. There is also a difference between the polar filaments of the two forms.

It is apparent from my description of the species that it seems to be the same as that described by Hahn found in the gill of fish of the

same kind. I propose for it, in accordance with my observations, the name *Myxosoma funduli*.

Ceratomyxa drepanopsettae Awerinzew

Habitat: The gallbladder of *Paralichthys dentatus*.

Seven out of fifteen individuals of about 30 cm. in length harbored the parasite.

Fantham and Porter (1912) made an observation on the effect of Myxosporidia upon the bile and gallbladder of fish. My observations are somewhat similar to the results mentioned by them. The wall of the infected gallbladder was usually thick and opaque. In this connection it may be interesting to mention one of my previous papers (1916). In the case of *Acheilognathus lanceolatum*, the wall of the gallbladder, highly infected with *Zschokkella acheilognathi* Kudo, was found rather thinner and more transparent in some cases than the normal ones, so that the parasites in the bladder and the duct could easily be seen from the outside. On the other hand, it was not rare to find an opaque gallbladder without any parasite in it. In two instances the gallbladder was only about two-thirds of that of other infected fish of similar length.

The bile of the normal fish was clear and greenish, while that of the infected ones was pale yellowish with a large amount of the floating mass, made up chiefly of degenerating epithelium, in which the parasites were found. The vegetative form found in the bile varies greatly in size and form, being sometimes slender and somewhat angular with three or four long fine pseudopodia, and sometimes flat and round, with or without pseudopodia (Figs. 8 to 11). It is not rare, however, to see specimens with a number of fine pseudopodia on all sides of the body (Fig. 8). Some of the pseudopodia attain a length of 30μ . Under the dark field microscope, the form changes very rapidly, probably on account of pressure and temperature. The parasites assume a round shape, withdrawing the pseudopodia (Figs. 10 and 11). A large number of very coarse granules fill up the protoplasm very thickly in almost all cases (Figs. 9, 10 and 11), so that differentiation of the protoplasm into ectoplasm and endoplasm could only be made in very young specimens.

Spores were seldom found in the bile when examined in a fresh condition. It was found, however, that if the bile was kept in small tubes in a refrigerator at about 5 to 15° C. for two or three days, rapid spore-formation took place. These are shown in figures 12 to 14. So far as I could discover, it is always disporous. The dimensions of the spore are: length, 8 to 10μ , breadth 64μ . The round polar capsules have a diameter of about 6μ . Owing to the scarcity of spores, my observation lacks details.

Of all the *Ceratomyxa* described up to the present time, *Ceratomyxa drepanopsettae* Awerinzew is nearest like the one under discussion. Awerinzew described the species as found in the gallbladder of *Drepanopsetta platessoides*. Later it was found by Auerbach in the gallbladder of *Pleuronectes platessa*, *P. flesus*, *Hippoglossus vulgaris*, and *Hippoglossoides limandoides*.

The dimensions are not given by Awerinzew. But the nuclear change in the vegetative form of his illustrations is very much like that of the present species. The probable dimensions of the spore given by Auerbach are about 12 to 14 μ in length and 56 μ in width, the diameter of the polar capsule being about 4 to 6 μ . The form of the spores illustrated by both authors is exactly like the present form. I conclude, therefore, that the two forms are identical.

Myxobolus sp.

Habitat: Spleen of *Perca flavescens*. Only one case of slight infection was encountered.

A very small number of isolated spores were found both in smear and section preparations. The form of the spore is ovoidal, attenuated at the anterior end. The coat is uniformly thick, having one polar capsule and a sporoplasm in which an iodophile vacuole could easily be detected. Two nuclei of the same size (about 2 μ) were usually present in the sporoplasm. The dimensions are as follows: Length and breadth of the spore 18 to 20 μ and 8 μ , respectively. The polar capsule is 7 to 9 μ long and 3 to 6 μ wide.

The species in question is probably identical with *Myxobolus pyriiformis* Thélohan described by him as found in the branchiae and spleen of *Tinca tinca* and kidney of *Misgurnus fossilis*.

I want to express my thanks to Dr. Noguchi for allowing me to carry on these observations in his laboratory.

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EXPLANATION OF PLATES

All the figures were photographed from fresh preparations under dark field illumination.

PLATE I. *Myxosoma funduli* n. sp.

Figs. 1 and 2. The infected gills.

Fig. 3. A group of spores.

Fig. 4. Optical cross section of a spore.

Figs. 5 and 6. Spores treated with perhydrol, showing the extruded filaments.

Fig. 7. A mechanically pressed spore with one of the filaments extruded.

PLATE II. *Ceratomyxa drepanopsettae* Awerinzew

Fig. 8. A relatively young vegetative form with fine pseudopodia.

Fig. 9. A large form, showing the outline of the nuclei and the granules.

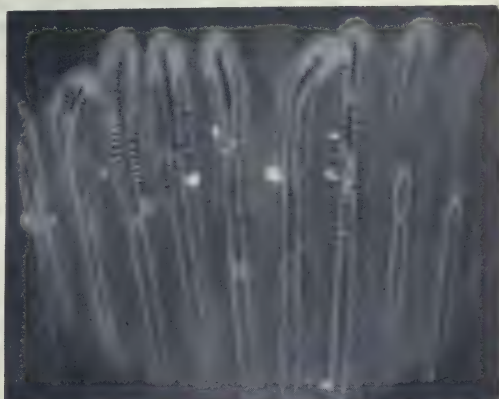
Fig. 10. A myxosporidium with a pseudopodium at one end of the body.

Fig. 11. The same specimen after 20 minutes, withdrawing the pseudopodium.

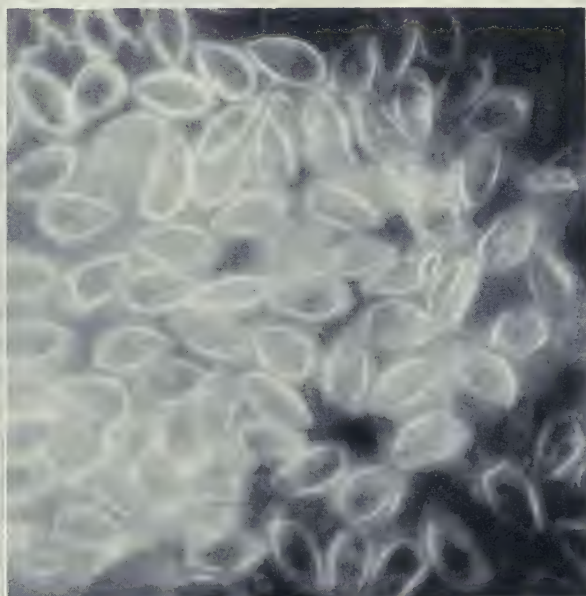
Figs. 12-14. Spores found in the bile after it had been kept in the refrigerator for three days.



1



2



3



4



5



6



7



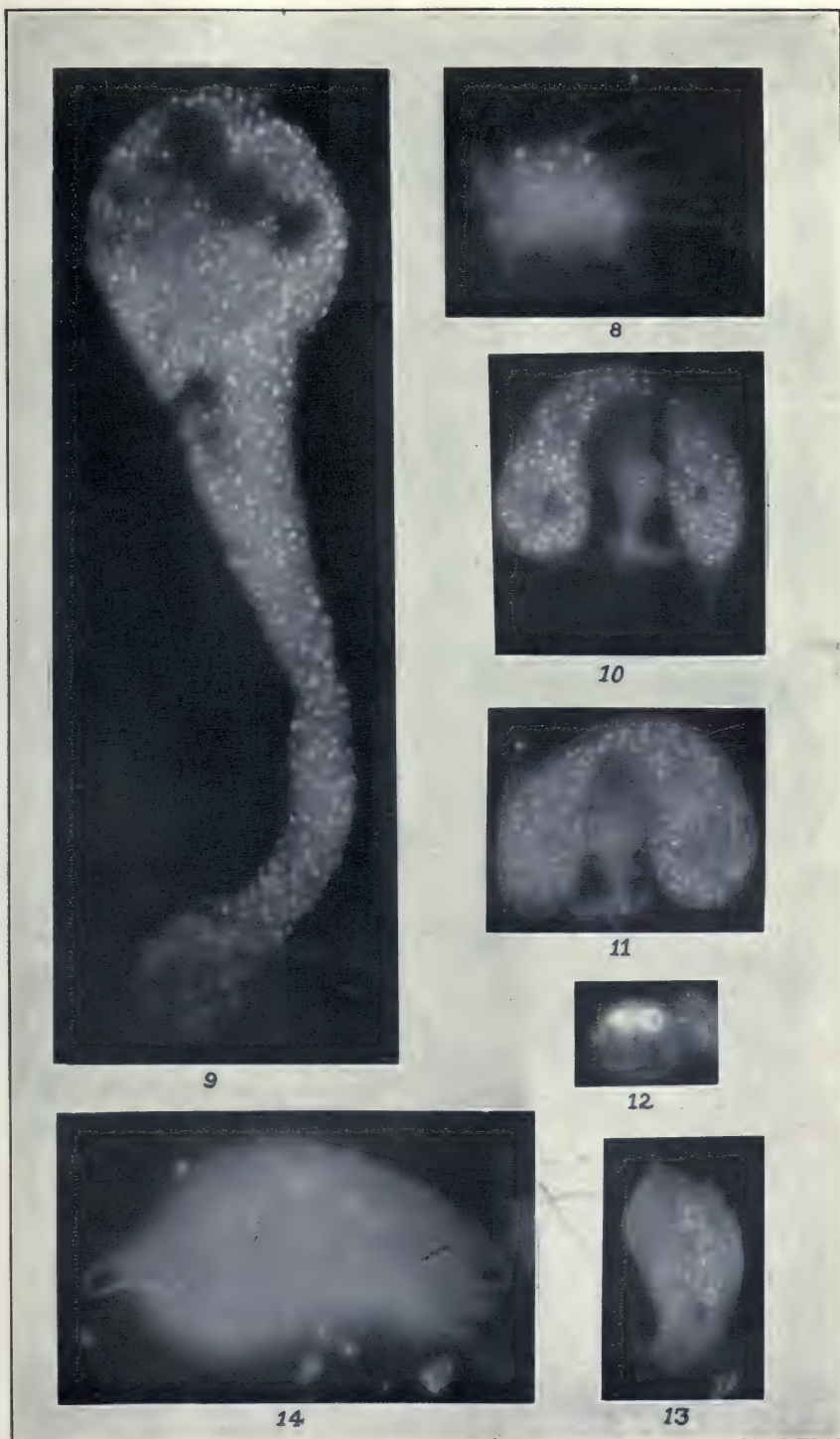


PLATE II



ACANTHOCEPHALA OF THE SUBFAMILY RHADINORHYNCHINAE FROM AMERICAN FISH *

H. J. VAN CLEAVE

But two species of *Acanthocephala* have, to the present time, been definitely ascribed to the genus *Rhadinorhynchus*. *Echinorhynchus pristis* Rud. was the original species for which Lühe (1911) created this genus. One year later he added another species to the genus by his description of *R. horridus* from Egypt. The older species has been reported as parasitic in fishes from various parts of the world. The descriptions given in these scattered reports differ so radically that it seems improbable that they could all apply to a single species. Here, as in other genera of *Acanthocephala*, it seems obvious that the older workers, recognizing the cardinal points which in modern classification have been accepted as indicative of generic value, have failed to observe the less obvious, though significant, differences which serve to separate species. Thus all members of the present genus *Rhadinorhynchus* have been identified as *E. pristis*, and the descriptions have usually been inadequate to enable more recent workers to recognize the same forms if encountered again. In some few instances the descriptions have been complete enough to permit one to recognize the forms described. Linton's work on *E. pristis* is of the type last mentioned.

In 1891 Linton recorded the occurrence of *Acanthocephala* which were at least similar to *E. pristis* from the intestine of *Tylosurus acus* (Lacép), and of *Lobotes surinamensis* (Bloch). After acknowledging "perplexity in attempting their identification," Linton tentatively ascribed some of his individuals to the species *E. pristis*, while others he referred to a new variety of that species. To the variety he gave the name *E. pristis tenuicornis*. In later work he remarked upon the presence in what he determined as *E. pristis* from fishes at Beaufort, N. C., of "a circle of hooks at the base of the proboscis which are longer than the other hooks" (Linton, 1908: 89). This "circle" of hooks was briefly mentioned in an earlier paper (1892: 531). In materials studied by the present writer, the spines at the base of the proboscis, though not forming a circle, are very conspicuous. After a comparison of these specimens with data given by Linton the writer is convinced that Linton was at various times dealing with at least two distinct species of the genus *Rhadinorhynchus* to which he gave

* Contributions from the Zoological Laboratory of the University of Illinois, No. 122.

reference as *E. pristis* and *E. pristis tenuicornis*. Further evidence of this will be presented in a later part of this paper.

In later works Linton described two additional species of Acanthocephala, *Echinorhynchus sagittifer* and *E. medius*, both of which obviously belong near to the genus *Rhadinorhynchus*. The former of these has been referred to Monticelli's genus *Echinogaster*, and in the present paper the writer includes the species *medius* in the genus *Rhadinorhynchus*. The records mentioned above constitute the only cases of *Rhadinorhynchus*-like forms reported from North America.

TABLE 1
COMPARISON OF *R. PRISTIS* WITH AMERICAN SPECIES OF RHADINORHYNCHINAE

Form	Proboscis Hooks		Body Spines		Embryos
	Number	Size	Arrangement	Size	
<i>R. pristis</i> (Rud) of Lühse, 1911	14 longit. rows of 26 hooks each	85 μ *	Scattered in two fields	110 μ *	95 \times 17 μ
<i>R. ornatus</i> n.sp. (<i>E. pristis</i> of Linton, 1892)	About 24 longit. rows of about 40 hooks each.	50-80 μ	Scattered	80 μ	60 μ long.
<i>R. tenuicornis</i> n.sp. (<i>E. p.</i> <i>tenuicornis</i> of Linton, 1892)	14 longit. rows of about 26 hooks each; crescent of spines at base of proboscis	♀40-80 μ ♂20 μ +	Scattered	♀60-80 μ ♂28 μ +	60 to 80 μ by 12 μ
<i>R. medius</i> (Linton, 1905)	About 22 longit. rows of about 20 hooks each	45-60 μ	Scattered	30-45 μ	75 \times 24 μ
<i>Echinogaster</i> <i>sagittifer</i> (Linton)	About 24 longit. rows of 15-18 hooks each	80 μ	Collar of scattered spines followed by 18-23 ventral cross rows	Collar 50-60 μ Ventral rows 60-70 μ	?

*Unfortunately Lühse has not given measurements of the hooks or body spines of *R. pristis*. The measurements given for *R. pristis* above are approximations obtained by finding the values for these structures on the basis of the magnification given with his figures. These can serve as mere approximations, for in checking over other drawings in the same article considerable error in magnification has been found. For example, 95 μ is the length given in the text for the embryos of *R. pristis*, while application of stated magnification to the embryo figured gives a value of but 83 μ for the length. Embryos usually vary some in length but Lühse's failure to state the range of variability in his measurements makes it impossible to calculate the probable error in his magnification.

Table 1 lists the points of difference and similarity between the forms dealt with in this paper. The writer has created a new species, *Rhadinorhynchus ornatus*, for the forms described by Linton as *E. pristis*. Specimens from the U. S. National Museum, examined by the writer, are apparently identical with Linton's *E. pristis tenuicornis*. After a careful study of these specimens, results of which have added some new data to our knowledge of the structure of the form, the writer has given a description of a new species, *Rhadinorhynchus tenuicornis*. In this connection it is interesting to note that

of the Rhadinorhynchinae but one species is known to Europe and one to Africa, while from the American continent four species representing two genera are now reported.

Genus RHADINORHYNCHUS Lühe 1911

Synonym: *Echinosoma* Porta 1907 (preoccupied), in part.

Generic Diagnosis.—Acanthocephala parasitic as adults in the intestine of fish. Anterior body region armed with scattered cuticular spines, ensheathed by cuticular folds. Proboscis and proboscis receptacle very long. Ventral proboscis hooks stronger than dorsal. Proboscis receptacle a two-walled muscular sac with the brain located near its middle. Lemnisci long, fingerlike.

Porta (1907:412) gave the name *Echinosoma* to a new genus of Acanthocephala which he created to include: *E. gibber* Olss., *E. vasculosus* Rud., *E. miliaris* Zenk., *E. roseus* Mol., *E. pristis* Rud., and several other species. The name *Echinosoma* is preoccupied and is for that reason not available for this group of Acanthocephala. Furthermore, the forms listed by Porta constitute a heterogeneous group which have but little in common aside from the presence of spines on the body. No species was cited as type of Porta's *Echinosoma*. On the other hand the first species mentioned in his list was *E. gibber* Olss. which has been quite commonly regarded as a synonym for *E. strumosus*. In 1904 Lühe created the genus *Corynosoma* with *C. strumosum* as type by original designation. Subsequently a number of new genera have been created which include species cited by Porta for his now disrupted genus *Echinosoma*. *Rhadinorhynchus* Lühe 1911, with *R. pristis* (Rud.) as type species, is one of this number. Consequently, *Echinosoma* Porta 1907 in part, must be regarded as a synonym of *Rhadinorhynchus* Lühe 1911.

Rhadinorhynchus ornatus nov. spec.

Synonym: *E. pristis* Rud. of Linton, 1892 and 1908. Text figure A.

Specific Definition.—With the characters of the genus *Rhadinorhynchus*, Lühe, 1911. Proboscis armed with from twenty-two to twenty-four longitudinal rows of about forty hooks each. Hooks on proboscis ranging from 50 to 80 μ in length. Anterior body region armed with scattered cuticular spines about 80 μ long. Embryos about 60 μ long.

Host: *Tylosurus acus* (Lacép.), Woods Hole, Mass.

The above definition is adapted from the original description by Linton.

Rhadinorhynchus tenuicornis nov. spec.

Synonym: *E. pristis tenuicornis* Linton, 1892. Figures 1 to 4 and text figure B.

The variety *Echinorhynchus pristis tenuicornis* created by Linton (1892), for what he considered a variety of the European species *E. pristis*, is clearly a distinct species. Table 1 presents the evidence of the distinctness of this and other forms dealt with in this paper. In elevating the variety to specific rank the writer has used the varietal name for the name of this species. Data given by Linton in his original description of the variety omitted several characteristics which are useful in drawing a closer limitation of the species. Fortunately, the present writer has had access, through the government collections, to additional specimens which in all essential details agree with Linton's description. A study of these specimens has made it possible to offer here a more complete description of *R. tenuicornis*.

Specific Definition.—With the characters of the genus *Rhadinorhynchus*. Proboscis armed with ten to fourteen longitudinal rows of approximately twenty-six hooks each. Proboscis hooks of female 40 to 80 μ long; of male near base may be as small as 20 μ . A conspicuous crescent of about seven long hooks on the ventral side of the proboscis at the region between neck and proboscis. Body spines of female 60 to 80 μ , of male about 28 μ . Embryos inside body cavity of female 60 to 80 μ long and 12 μ wide, with middle membrane drawn out into attenuated polar capsules.

Hosts: *Tylosurus acus* and *Lobotes surinamensis* at Woods Hole, Mass., and "trout" at Baltimore, Md. The collection of specimens from this last host was made by Hassall in October, 1891. Specimens are on deposit in the Hassall collection of the U. S. National Museum, catalog number 6324.

The anterior body region in this species, especially among the females, tapers considerably to reach the size of the proboscis at the point of insertion with the proboscis receptacle. In many specimens the extremely long proboscis and the anterior region of the body are withdrawn within the body. The proboscis receptacle is so long that in retracting the inverted proboscis toward its base much of the anterior body region also becomes inturned. Linton in 1891 and again in 1905 mentioned the "circle of prominent arcuate hooks" at the base of the proboscis. In *R. tenuicornis* the present writer has found a group of prominent hooks in the same locality, but they take the form of a crescent instead of that of a circle, since in their distribution they are restricted to the ventral surface of the proboscis. This arrangement in the form of a crescent is shown clearly even in specimens which have the proboscis and anterior body region inturned (Fig. 2).

These hooks differ in appearance from the remainder of the proboscis hooks and also differ from the body hooks. They present a peculiar granular appearance in stained whole mounts.

Rhadinorhynchus medius (Linton)

Synonym: *Echinorhynchus medius* Linton, 1908.

This species was described by Linton (1908:88) from Bermuda fishes. In his description he called attention to the fact that "this species is near *E. pristis*, in external appearances," but in listing the points of difference he omitted several of the most essential. The present writer has reexamined the type material deposited in the U. S. National Museum and has been able to corroborate Linton's original description as far as it goes. A brief summary of the diagnostic characters of the species follows:

Specific Definition.—With the characters of the genus *Rhadinorhynchus*. Proboscis linear to fusiform, armed with about twenty-two longitudinal rows of about twenty hooks each. Hooks near base (basal two or three of each row) about 45μ long, remainder of hooks fairly uniform in size, about 60μ long. Hooks deeply embedded in cuticula, recurved, stout. Neck smooth, conical. Body spines 30 to 45μ long; extend on ventral side of body from just back of neck to about one-third the length of the proboscis receptacle; on dorsal surface extend only about one-half the distance of ventral. Embryos 75μ long by 24μ wide.

Host: *Mycteroperca apua*, in intestine. Locality, Bermuda Islands. Larvae in cysts on viscera of various fishes. Types deposited by Linton in U. S. National Museum, catalog number 5796.

Genus ECHINOASTER Monticelli 1905

Synonyms: *Echinorhynchus* in part. *Echinosoma* Porta, 1907, in part.

Monticelli (1905:11) in a footnote created the genus *Echinogaster* giving a six-word diagnosis and designating no type for this or for either of the other two genera (*Pomphorhynchus* and *Chentrosoma*) created at the same time. Porta (1907:413) reduced *Echinogaster* to a subgenus of his newly created genus *Echinosoma*, and referred *E. sagittifer* of Linton to *Echinogaster*. The name *Echinosoma* is preoccupied and can consequently not be accepted as a name for Porta's genus. Furthermore, the present writer is definitely of the opinion that the genus *Echinogaster* was erroneously reduced to subgeneric rank, and here proposes elevating it again to full generic standing. Lühe (1912:278) called attention to the close relationship which exists between members of the genera *Rhadinorhynchus* and

Echinogaster and proposed that a new subfamily, the Rhadinorhynchinae, be erected in recognition of this fact. Many points in the structure of the members of the genus Echinogaster are unknown at the present time, consequently it seems advisable to offer as a generic diagnosis only those points which serve as a ready means of separating Echinogaster from Rhadinorhynchus. A more complete diagnosis can be given only upon a restudy of forms belonging to this genus.

Generic Diagnosis.—Rhadinorhynchinae with ventral cross rows of body spines.

Echinogaster sagittifer (Linton, 1889)

Synonyms: *Echinorhynchus sagittifer* Linton, 1889. *Echinosome* (*Echinogaster*) *sagittifer* (Linton) Porta, 1907.

Specific Definition.—Rhadinorhynchinae with characters of the genus Echinogaster. Proboscis clavate, bluntly rounded in front, increasing slightly for a short distance back from the tip, then narrowing gradually to the base; armed with about twenty-four longitudinal rows of about fifteen to eighteen hooks each. Longest proboscis hooks about 80μ long. Body spines arranged in two distinct groups—a collar of spines 50 to 60μ long on the body region just behind the neck, and eighteen to twenty-three series of ventral transverse rows of spines each containing from six to twenty-four sagittate spines about 60 to 70μ long. Measurements of embryos not given.

Host: *Rachycentron canadus*, in intestine, at Beaufort, N. C. Larvae encysted in mesentery and viscera of various marine fishes.

Key to the species of Rhadinorhynchinae from American fish.

- 1 (6) Rhadinorhynchinae with scattered body spines.....2
- 2 (5) Body spines of females over 60μ long.....3
- 3 (4) Proboscis with less than sixteen longitudinal rows of hooks
..... *Rhadinorhynchus tenuicornis*
- 4 (3) Proboscis with more than twenty longitudinal rows of hooks
..... *Rhadinorhynchus ornatus*
- 5 (2) Body spines of female less than 50μ long.....
..... *Rhadinorhynchus medius*
- 6 (1) Body spines on ventral surface arranged in cross rows....
..... *Echinogaster sagittifer*

SUMMARY

A new species, *Rhadinorhynchus ornatus*, is created for *E. pristis* of Linton, 1891.

Rhadinorhynchus tenuicornis nov. spec. is created for Linton's variety *E. pristis tenuicornis*.

Echinosoma Porta, 1907, is preoccupied and in part is to be considered as a synonym of *Echinogaster* Monticelli, 1905.

Echinorhynchus medius Linton, 1908, is assigned to the genus *Rhadinorhynchus*.

A key is given to the *Rhadinorhynchinae* of America including three species of the genus *Rhadinorhynchus* and one species of *Echinogaster*.

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EXPLANATION OF FIGURES

Text Figures A. and B. showing distinctive differences in proboscis armature of *R. ornatus* and *R. tenuicornis*.

(A.) *R. ornatus*, median region of proboscis of female; \times about 150. From Linton, 1891, Fig. 33.

(B.) *R. tenuicornis*, portion of proboscis of male, \times about 150. From Linton, 1891, Fig. 53.

EXPLANATION OF PLATE

All drawings made with the aid of a camera lucida. Magnification is indicated by the reference line accompanying each figure which has the value of 50μ .

Rhadinorhynchus tenuicornis nov. spec.

Abbreviations used: *a*, anterior; *br*, brain; *lem*, lemnisci; *p*, posterior; *pr*, proboscis receptacle.

Fig. 1.—Anterior body region of male with partially inverted proboscis. Basal crescent of hooks is wanting in this specimen. The proboscis had been slightly damaged at the point where they should occur.

Fig. 2.—Optical section of female in anterior ventral region of body showing group of hooks at base of inverted proboscis.

Fig. 3.—Surface view of anterior body region of female with proboscis completely inverted.

Fig. 4.—Embryo from uterus of mature female.

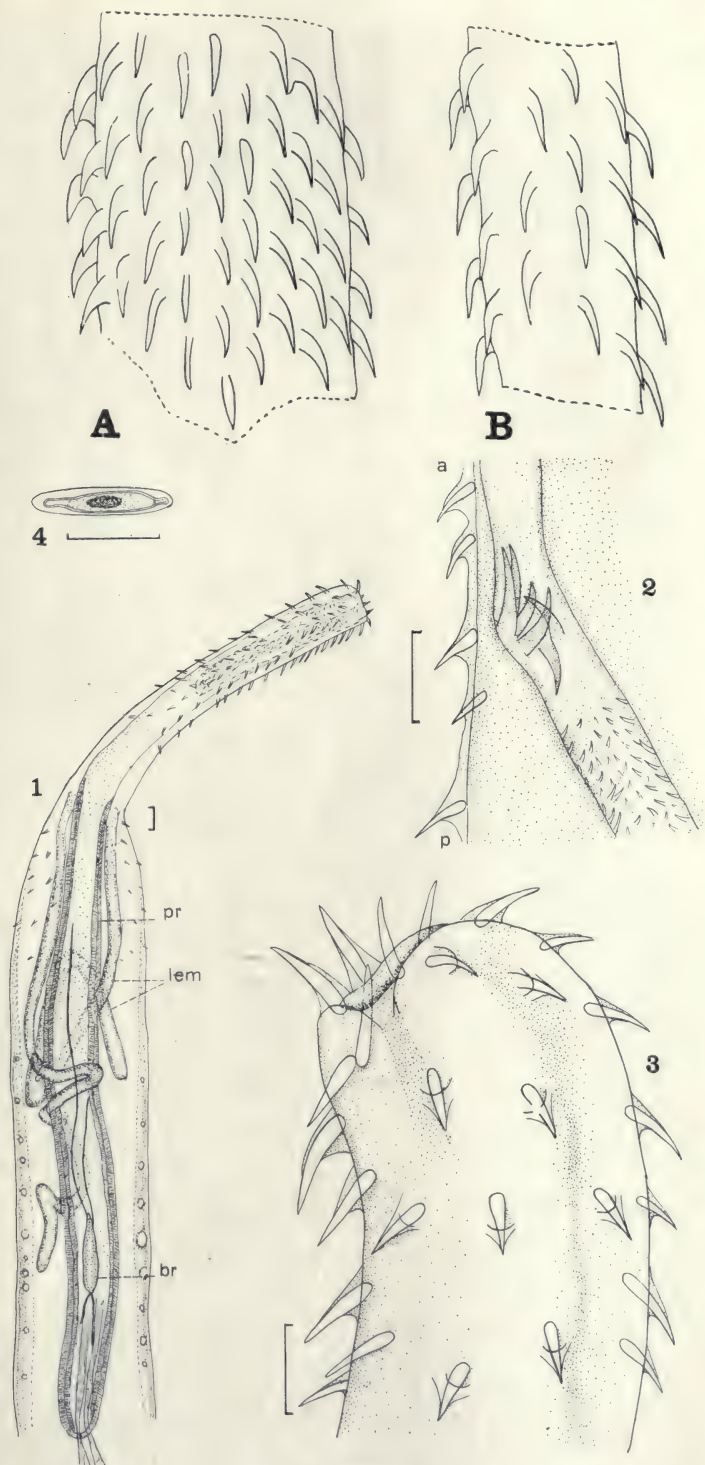


PLATE III



NOTES ON TWO SPECIES OF NEMATODES [*GONGYLONEMA INGLUVICOLA* RANSOM, 1904, AND *CAPILLARIA STRUMOSA* (REIBISCH, 1893)] PARASITIC IN THE CROP OF CHICKENS

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In making postmortem examinations of Philippine chickens, two species of nematode worms have been found in the walls of the crop. Both of them live in winding burrows in the mucosa and are never found free in the lumen. The larger of the two forms which was found in about 40 per cent. of all the chickens examined, has been identified as *Gongylonema ingluvicola* Ransom, 1904. The second and more slender species I have recognized as *Capillaria strumosa* (Reibisch, 1893). It was found in about 30 per cent. of the chickens dissected.

Gongylonema ingluvicola Ransom, 1904

This species was described by Ransom from the crop of a chicken from Key West, Florida. It is the only species of this genus which has been described from birds. On superficial examination of my material I was inclined to believe that the Philippine species was different from *ingluvicola*; however, after a careful study of a number of specimens and a comparison of the measurements of the different parts of the body with Ransom's description, I do not feel justified in separating this form from that species.

The body is white in color and the cuticula is annulated, the annulations measuring 10 to 11 μ in breadth in the females and 6 to 7 μ in the males. The arrangement and extent of the "cuticular bosses" at the anterior end of the body in my specimens (see Fig. 1) is almost identical with Ransom's description and figure. The "transversely elongated, large, plate-like shield" through which the excretory pore opens seems, however, to be considerably wider in my specimens than is indicated in Ransom's figure, and projects considerably from the surface of the body. The lateral membranes, which start a short distance back of the cervical papillae, are short and inconspicuous.

The following table shows graphically the measurements of Ransom's original specimens as compared with the Philippine form. My measurements are the maximum and minimum of seven adult females and the same number of males.

TABLE 1

Gongylonema ingluvicola	Male		Female	
	Ransom	Wharton	Ransom	Wharton
Length of body.....	17-19 mm.	17-20 mm.	32-45 mm.	40-55 mm.
Breadth of body.....	250 μ	224-256 μ	400-490 μ	320-420 μ
Extent of cuticular bosses.....	575-680 μ	480-496 μ	1.3-2.6 mm.	1-1.36 mm.
No. of rows of bosses.....	16	?	20-24	20-24
Distance of cervical papillae from anterior end.....	100 μ	100-125 μ	135 μ	120-150 μ
Distance of excretory pore from anterior end.....	300 μ	368-400 μ	450 μ	450-480 μ
Length of anterior part of esophagus	280-400 μ	400-480 μ	540 μ	575-608 μ
Length of posterior part of esophagus	3.2-3.3 mm.	3.2-3.3 mm.	5-6 mm.	4.5 mm.
Distance of anus from tip of tail..	225-275 μ	264-275 μ	165-215 μ	240-288 μ
Distance of vulva from tip of tail.....			2.5-3.3 mm.	3-3.5 mm.
Length of vagina.....			13 mm.	11-14 mm.
Eggs in vagina.....			50 x 36 μ	52.9 to 56.7 x 37.8 μ
Width of tail, including alae.....	225 μ	208-240 μ		
Length of right ala.....	500-575 μ	560-736 μ		
Length of left ala.....	600-700 μ	720-800 μ		
Left spicule	17-19 mm. by 9 μ	17-19 mm. by 7-9		
Right spicule	100 x 15 μ	120 x 20 μ		

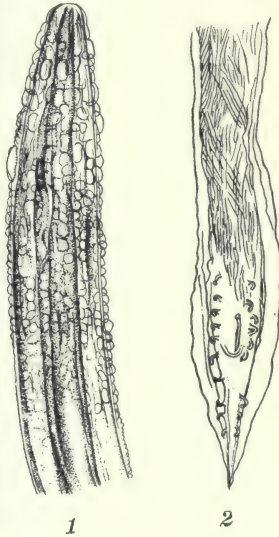


Fig. 1.—Lateral view of anterior end of female *Gongylonema ingluvicola*.
Fig. 2.—Posterior end of male showing regular arrangement of genital papillae and the right spicule. (Drawings by Castro. Camera lucida outlines.)

The number and arrangement of the genital papillae of the male, which is generally very regular in members of this genus, show marked variations in this species. Ransom says "The number of genital papillae is rather variable and they are not symmetrically arranged. In the specimens examined the number of preanal papillae on the left side was 5 to 7, on the right side 4 to 4, and the number of postanal papillae 3 to 4 on the left side, 4 on the right side." I have carefully studied the papillae in 14 of my specimens and found the number as follows:

TABLE 2.—ARRANGEMENT OF ANAL PAPILLAE

	Preanal		Postanal		Total
	Rt.	Lt.	Rt.	Lt.	
No. 12.....	7	6	4	4	20
No. 1.....	6	6	4	4	20
No. 2.....	6	6	4	4	20
No. 4.....	6	6	4	4	20
No. 10.....	6	6	4	4	20
No. 11.....	6	6	4	4	20
No. 14.....	6	6	4	4	20
No. 5.....	5	5	5	5	20
No. 6.....	5	5	5	5	20
No. 3.....	5	5	5	4	19
No. 9.....	5	5	4	4	18
No. 7.....	6	2	4	4	16
No. 8.....	4	6	3	2	15
No. 13.....	0	7	3	3	13

In Specimens 1, 2, 4, 10, 11, 14, 5, 6 and 9 the papillae are arranged symmetrically in pairs (see Fig. 2). In the other specimens which appear to be asymmetrical, this appearance is the result of the suppression of one or more papillae on a side as in Nos. 3, 7 and 8, or the addition of an extra papillae anteriorly as in No. 12. It would require the study of a much greater series than I have in my possession at the present time to determine whether or not ten pairs of papillae is the normal number, but the preponderance of this number and arrangement in my series is, I think, worthy of note. The slightly greater length, in my specimens, of the lateral alae as shown in Table 1 can be accounted for by the greater body length. The size and shape of the spicules is practically the same as in Ransom's specimens. The extreme length of the left spicule is a characteristic which easily distinguishes this form from other species of the genus.

Geographical distribution. All of my specimens have come from chickens which were raised in or very close to Manila, so I am not able to state whether this form is of general distribution in the Islands.

Capillaria strumosa (Reibisch, 1893)

This species has been reported from *Phasianus colchicus* and *Gallus domesticus*. On account of their extreme slenderness and the tortuous windings of their burrows it is very difficult to obtain whole specimens. I have several whole females, but have not succeeded in obtaining any complete males.

The length of the females runs from 40 to 55 mm., and they are 100 to 120 μ thick. The males are estimated to be from 17 to 25 mm. long and 70 to 80 μ in thickness. The cuticula is striated transversely and the cephalic extremity is surrounded by the characteristic cuticular dilation. The oesophagus in the female is from 7 to 8 mm. long and the vaginal opening is situated just at the end of the oesophagus. The eggs in the vagina measure 60 to 64 μ by 26 to 28 μ . The male is provided with two projections around the anal opening.

Neither one of these forms is often found in any great numbers in the chickens I have examined, and as far as I could see they do not cause any pathological conditions of importance.

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TWO NEW NEMATODES COMMON IN SOME FISHES OF CAYUGA LAKE*

MEYER WIGDOR

Very little work has been done in this country on the nematode parasites of fishes. Suitable monographs are available on the nematodes of rodents, ruminants and some domesticated animals, but when the identification of fish nematodes is attempted, resort must be had to foreign literature such as the key to the nematodes in Brauer's *Süsswasserfauna Deutschlands*, Railliet and Henry's many valuable papers, etc. Ward's recent compilation (1918) of freshwater nematodes in "Ward and Whipple's Freshwater Biology," is a very important and valuable aid in the identification of these forms and will undoubtedly give an added impetus for further study along these lines, but much work remains to be done before a really comprehensive monograph on nematodes from freshwater fishes will be possible.

That the study of fish nematodes presents a large and little investigated field in this country is evidenced by the number of new genera and species recently established by workers in this group of fish parasites. Ward and Magath (1917) describe eight new species of nematodes from freshwater fish, three constituting new genera and five agreeing sufficiently with European forms to be listed in already existing genera. The two new species described in this paper both fall within existing genera, one being placed in the genus *Rhabdochona* in the family *Thelaziidae* of the superfamily *Spiruroidea*, which superfamily seems to hold a very prominent place among the parasites of freshwater fishes, and the other being placed in the genus *Hysterothylacium* in the family *Heterocheilidae* of the superfamily *Ascaroidea*.

Rhabdochona cascadilla Wigdor nov. spec.

Rhabdochona is a new genus created by Railliet (1916) in the family *Thelaziidae* of the superfamily *Spiruroidea*. He characterizes the family as comprising forms possessing a head, either naked or provided with cuticular expansions or with a cup-shaped covering; the mouth without lips or only two in number and followed generally by an elongated vestibule or a short buccal capsule.

He characterizes the genus as follows: Mouth with two lips, limiting a funnel-shaped cavity which is supported by longitudinal cuticular ribs. Esophagus of medium length and with two distinct parts. Male

* Contribution from the Entomological Laboratory of Cornell University.

with a conical tail, pointed and recurved. No caudal alae; numerous simple preanal and postanal papillae. Two unequal spicules. Female with a straight conical, elongated tail. Vulva towards the posterior end of the body. Uteri divergent. Habitat: intestine of freshwater fishes. Type species: *Dispharagus denudatus* Duj. 1845.

In Cascadilla creek, especially at Dwyer's pond, *Rhabdochona cascadilla* was found to be especially common in the small intestine of the horned dace, *Semotilus atromaculatus* (Mitchell) and the cayuga minnow, *Notropis cayuga* (Meek), two minnows especially common in this tributary to Lake Cayuga.

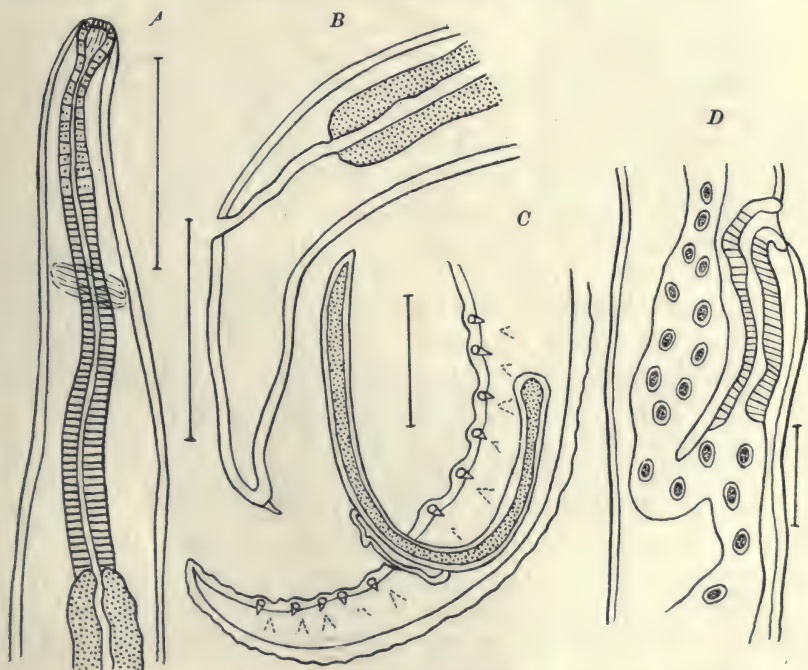
This new species may be briefly characterized as follows: Body filiform, cylindrical, attenuated at anterior end. Head truncated, somewhat rounded and smooth. Mouth possessing two lips terminating a funnel-shaped cavity which is supported by longitudinal cuticular ribs bearing two small, somewhat conical, papillae (Fig. 1). Esophagus distinctly short and made up of an anterior and posterior portion, the former being approximately one-third the length of the latter.

Male: 3.01 to 4.11 mm. in length and 0.096 to 0.104 mm. in width at widest part. Length of esophagus 0.24 mm. Distance of nerve ring from anterior extremity 0.115 mm. Spicules very prominent. Large spicule 0.04 mm. in length, pointed and very flexible and generally U-shaped when protruding from body. Small spicule slightly more than one-fifth as long as the large spicule and provided with a blunt, cap-like distal portion. Tail conical, subacute and recurved. Six pairs of preanal, one pair of adanal and five pairs of postanal papillae (Fig. 2).

Female: 6.80 to 9.28 mm. in length and 0.096-0.128 mm. in width at widest part. Young immature females 3.8 to 4.06 mm. in length and 0.08 to 0.096 mm. in width. Vulva a transverse slit in posterior portion of the body about five-eighths the total body length from anterior extremity. Ovijector extends 0.18 mm. posteriad of the vulva. Uteri divergent (Fig. 3) and very voluminous, full of developing ova 32 by 16 μ in diameter, and filling up approximately three-quarters of the body cavity. Uterus bifurcates 0.272 mm. posteriad of the vulva, one branch extending anteriad, the other posteriad. Anterior ovarian tube reaches 2.42 mm. anteriad of the vulva, and then loops posteriad for a distance of 1.019 mm. Posterior ovarian tube extends to a point 0.586 mm. from posterior extremity and then loops about and extends anteriad for a distance of 1.764 mm. Anus 0.06 mm. from posterior extremity. Tail characteristically very blunt, terminating in a spine-like process and usually bent back at an angle to the body (Fig. 4).

Hysterothylacium cayugensis Wigdor nov. spec.

A nematode found very commonly in the pike, *Esox lucius* L, and much less commonly in the bullhead, *Ameiurus nebulosus* (LeSueur), in the waters of Lake Cayuga, in all probability falls in the new genus described by Ward and Magath (1917) as *Hysterothylacium*. This is in the family Heterocheilidae which they characterize as follows: "Body without anterior tunic but with narrow lateral alae ("wings"). Lips three, not prominent. Esophagus arising from anterior end of



All figures are camera drawings. The reference line is 100 μ long in each case.

Fig. 1.—*Rhabdochona cascaddilla*; A, Anterior extremity. B, Posterior extremity. C, Posterior extremity of male showing specules and papillae. D, Female, showing vulva, ovejector and divergent uteri.

intestine, directed posteriad." These workers have found the males only, the females being unknown, while the large number of specimens I have obtained are all unfertilized females.

Hysterothylacium cayugensis may be briefly characterized as follows: Length, 15 to 20 mm.; width, 0.14 to 0.19 mm. Three pairs of well-defined lips (Fig. 5). Head without anterior cuticular expansions. Esophagus long, averaging 2.35 mm. in length and 0.08 mm. in width. Distance of nerve ring from anterior extremity 0.21 mm. The esophagus is followed by what appears at first sight to be an

esophageal bulb, but on closer examination this seems to be a rotund dilation of the cecum which arises from the anterior portion of the intestine and extends posteriad as a cecum. This bulb-like expansion of the cecum is apparently glandular in nature, which would preclude the idea that this is an esophageal bulb. The expansion measures 0.128 mm. in diameter, while the cecum, including the dilation, is quite long, measuring 0.68 mm. in length and 0.026 mm. in width. Broad lateral alae (Fig. 6), width nearly one-fourth the diameter of the body, extend from base of lips to base of esophagus or farther. Body with marked transverse striations 2μ apart.

Male unknown.

Female with vulva in anterior portion of body, at a point approximately two-fifths of the length of body from the anterior end. Ovijector quite long, extending 0.549 mm. posteriad of the vulva. Uterus forks (Fig. 7) 0.588 mm. posteriad of the vulva to form two divergent uteri, one branch extending anteriorly, the other posteriad. Uteri long, looping transversely and diagonally (Fig. 8) through a large portion of the body cavity. Posterior ovarian tube extends to 0.14 mm. from the anus, which in turn lies 0.252 mm. from posterior extremity. Posterior extremity usually very acute.

Although this species has been placed in the genus *Hysterothylacium*, it differs essentially from Ward and Magath's description of the genus in the following particulars: There is no esophageal bulb, but a glandular rotund expansion of the cecum suggesting a bulb; and the cecum is quite long instead of short. In *H. brachyurum*, Ward and Magath's species, the cecum is approximately one thirty-third of the total length of the body; in *H. cayugensis*, Wigdor's species, it is approximately one twenty-fifth of the total length. The lips are well-defined in *H. cayugensis*. Ward and Magath (1916) state that the lips are not prominent in their species.

In assigning this species to the genus *Hysterothylacium*, the writer has assumed that in view of the features in common, it is possible that what Ward and Magath regard as an esophageal bulb, and what I regard as a proximal dilation of an intestinal cecum, are identical structures. If this assumption is correct, the two species are congeneric. Their specimens being all males and mine all females, the two might even have been regarded as identical species, if it were not for the fact that their males attained the size of 32 mm., while the largest of my females attained a maximum length of 20 mm. Since the female nematode is almost invariably larger than the male of the same species, this is excellent evidence of the specific distinctness of *H. cayugensis*. If, however, Ward and Magath are correct in their interpretation of the structure as an esophageal bulb, the form

described here must be transferred to another genus and may require the erection of a new genus.

Besides the mature worms, immature forms in the larval stage were obtained from the pike. They were 6 to 10 mm. in length and approximately 0.1 mm. in width, all of them as far as could be determined, being immature females.

Further studies on the minnows and small immature fish of Cayuga Lake disclosed forms which are undoubtedly the early larval stages of this worm. The presence of lateral alae, and a posteriorly directed intestinal cecum, with a spherical anterior expansion, and mouth parts bearing a strong resemblance to the mature form, shows that it is the same worm that reaches maturity in the pike and bullhead, after being ingested by the latter in eating the intermediate hosts. The host fishes

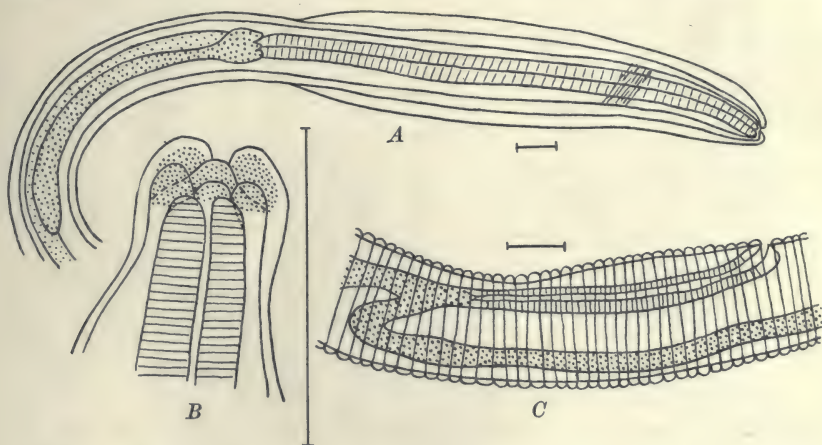


Fig. 2.—*Hysterothylacium cayugensis*; A, Anterior extremity showing lateral alae, esophagus, cecum and intestine. B, Anterior extremity showing lip arrangement. C, Female, showing vulva, ovejector and divergent uteri.

of this early immature stage are: golden shiner, *Abramis chrysoleucus* (Mitchell); satin-fin shiner, *Notropis whipplei* (Girard); blunt-nosed minnow, *Pimephales notatus* (Rafinesque); barred killifish, *Fundulus heteroclitus* (Linn); Cayuga minnow, *Notropis cayuga* (Meek); common sun-fish, or pumpkin-seed, *Eupometis gibbosus* (L), and the common white sucker, *Catostomus commersoni* (Lacépède).

The size of the forms in the various hosts are as follows:

	Length mm.	Width mm.
Barred killifish	1.80 to 3.10	0.064 to 0.082
Blunt-nosed minnow	1.71 to 4.40	0.051 to 0.124
Cayuga minnow	1.65 to 3.10	0.048 to 0.082
Golden shiner	1.09 to 1.94	0.053 to 0.070
Satin-fin shiner	1.38 to 2.12	0.048 to 0.072
Sucker	1.16 to 2.09	0.048 to 0.072

The writer wishes to express his most grateful acknowledgment and appreciation to Dr. William A. Riley and to Dr. Maurice C. Hall for invaluable help in connection with the investigation and the preparation of the foregoing paper.

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THE DEVELOPMENT OF GREGARINES AND THEIR
RELATION TO THE HOST TISSUES: (II) IN
CEPHALOIDOPHORA DELPHINIA (WATSON)*

MINNIE WATSON KAMM

This paper is the second of a series in which the writer aims to depict the consecutive stages through which gregarines of various genera pass in becoming mature sporonts, and the relation, whether deleterious or not, which the parasite bears to the intestinal epithelium from which it receives its nourishment. The former paper (Kamm, 1917) contained a brief historical survey of previous investigations of a similar nature.

The parasite chosen in the present instance is *Cephaloidophora delphinia* (Watson), first described by the writer in 1916, the host being the large white beach flea, *Talorchestia longicornis* (Say), taken at Cold Spring Harbor, Long Island, in the summer of 1915. The alimentary tract was removed intact, fixed and preserved. Sections were cut at 4μ and stained with Delafield's hematoxylin. Parasitism was found to occur in more than 30 per cent. of the 260 intestines examined for live sporonts (Watson, 1916); even where gregarines are present, it is rare to find more than a dozen sporonts, and very few intracellular stages in a single host. It is almost by accident that a good infection is encountered.

The alimentary tract of the Gammaridea, a suborder of the Arthropoda, consists (Calman, 1909) of the stomodeum, a long mid-intestine, and a short proctodeum. In my preserved material, most of the stomodeum is missing, having been severed with the head of the flea. At the junction of stomodeum and mid-intestine in *Talorchestia* (Fig. 1) is found one pair of hepatic caeca longer than the rest of the alimentary tract and opening into the same by two ducts. At the union of mid-intestine and proctodeum occurs one pair of caeca opening into the alimentary tract by separate ducts. These caeca are about two-thirds the length of the mid-intestine, looped upon themselves, and finally extend down the proctodeum loosely attached at its side. How they open to the exterior is not determined, as the intestines were clipped off at the posterior end. The caeca are probably excretory in function; they are filled with calcareous concretions for hastening the hardening of the exoskeleton after moults. The proctodeum in *Talorchestia* is nearly as long as the mid-intestine. The former is much thinner-walled than the latter. From the intestinal content one

* Contributions from the Zoological Laboratory of the University of Illinois, No. 123.

sees that the flea feeds upon algae and decaying wood and insects, and it probably finds additional organic matter in dead crabs and fish.

The epithelial cells of the mid-intestine are thrown into fan-shaped lobes (Fig. 6); the individual cells are columnar and packed closely together, and are heavily ciliated on their free surfaces. They are rich in protoplasm, which is either fairly evenly distributed and lightly reticulate or abundant only in the coelomic portion, that part nearer the lumen often being vacuolated. In the proctodeum, where absorption is diminished, the cells are short and broad, more nearly cubical in shape, and the protoplasm is coarsely reticulate. A spiny chitinous lining is present here.

It is in the mid-intestine only that the young parasites find their temporary lodging place. Obviously, the mature free sporonts find conditions at an optimum in the same region where the absorption of chyle is at its maximum. Where sections reveal the presence of sporonts in the lower regions, it is probable that the parasites are being swept down on the periphery of compact food-masses, and are on their way to speedy destruction as the feces are formed and ejected. The mature sporonts, then, collect between the food-masses and the lumen of the mid-intestine for protection, and here also they are in the richest food belt (Fig. 9).

It is a noteworthy fact that no parasites have been found in the hepatic caeca, for in several species studied from insects parasites are found here almost as freely as in the intestine itself. No parasites were found outside the coelomic layer of the epithelium or boring their way through this layer, as was noted in *Stenophora lactaria* (Watson, 1916). Gametes taken from immature developing cysts do not differ in size, but there is a slight difference in staining reaction, indicating a possible sexual differentiation (Watson, 1916). Spores have not been seen to date, neither has the sporozoite been found, and therefore the first stage in intracellular development cannot be shown. Gregarines in all stages of development stain perfectly homogeneously, and for this reason are easily distinguished from surrounding tissue, even in very young stages.

The first stage seen in my sections (Fig. 2) indicates a tiny ovoidal unilocular intracellular parasite smaller than a normal cell-nucleus and situated near the base of the cell which has been penetrated. The trophozoite has begun to absorb nourishment from the cell, which is already vacuolated in the region of the parasite and the nucleus, being nearby, has already begun to shrivel from absorption and probably also from the effect of the parasite excretions. The cell shows no hypertrophy in this early stage.

In a slightly later but still septumless stage of development (Fig. 3) there is also no evidence of hypertrophy, but the originally parasitized cell and the two contiguous ones now show distinct signs of degeneration, the walls in the proximity of the parasite being less clearly defined and the protoplasm more vacuolate than in other parts of the cells. The nuclei have changed from ovoidal to irregularly dolioform in shape.

Figure 4 shows sections of somewhat later stages with more advanced changes in the destruction of the host cells. Where still remaining, nuclei of destroyed cells have shrivelled and stain very darkly, the chromatin granules only being left, and the outlines of the cells have become indistinct throughout.

The next stage (Fig. 5) is that of a small parasite in which a septum is formed dividing it into protomerite and deutomerite. These two parts already show slightly different staining reactions, the former, although containing fewer protoplasmic granules taking the color more readily, a feature characteristic of the parasite throughout the remainder of its life. A small papilla is now visible at the apex of the protomerite which is retained in the adult. Léger and Duboscq (1911) mention this as a "rudimentary structure" and Mercier (1912) calls it an epimerite. It is homologous with a similar structure in *Stenophora lactaria* (Kamm, 1917: 126), and both are obviously functionless. As stated for the one previously described, this structure is probably the vestige of an organ which was useful and functional in the past; and this theory would lead to the conclusion that gregarines without or with only vestigial epimerites represent a higher stage of development than forms with a true functional epimerite in the cephalont stage.

Numerous instances have been found in which the parasite has located itself at the base of a lobular group of cells, and in growing is absorbing nourishment from the whole group at once—an arrangement highly advantageous to the parasite, but very destructive to the host tissues. In Figure 6 is shown (a) the cross-section of a small gregarine so located. The outline of that portion of the cells nearest the parasite is lost, but parts nearer the lumen still retain much of their individuality. The nuclei in the latter region are still but little affected, but if the nucleus happens to be situated near the parasite it is affected and soon becomes absorbed completely. This proves that nuclei in general are not more susceptible to the presence of the parasite than is the vegetative protoplasm of the cell. The cell-wall next the lumen and the ciliary boundary are the last to lose their identity and often when the entire cell-content has disappeared there is still considerable of the lobular contour left (Fig. 7). No hyper-

trophy is present, the apparent enlargement of the tissue in which the parasite is located being merely the normal contour of the lobules. Thus it is seen that the cells affected by the parasite do not become hypertrophied at any stage, but from the first are absorbed. Therefore, it is apparent that excretions of the gregarine do not seriously affect the cell protoplasm.

Léger and Duboscq (1911) speak of development at first as intracellular and later as intercellular; I should, however, scarcely call the position of parasites of this particular species, after many cells have been totally or partially destroyed, as intercellular.

That the gregarine may occupy any position within the epithelium is indicated in Figure 6, as at *a* it lies horizontal with the basal muscular layer and parallel with relation to the anterior-posterior direction of the intestine; in *b* it lies obliquely; in *c* it lies at right-angles to the long axis of both the intestine and the epithelial cells and in Figure 7 it is seen to lie perpendicular to the long axis of the intestine and parallel to the long axis of the cells. Mercier (1911) mentions that in *Cephaloidophora talitri* the position of the parasite within the cell is not constant. This diversity of position is quite in contrast to that in *Stenophora lactaria*, in which the parasite in almost every instance lay with the protomerite toward the muscular layer at all stages of its development (Kamm, 1917: 126). The explanation for this difference between the two species probably lies in the fact that in *S. lactaria* the parasite is crowded and has no room for movement, while in *C. delphinia* it has considerable freedom for rotation. In its growth *S. lactaria* rarely uses up more than a few cells, while *C. delphinia*, lying at the base of a whole group, uses protoplasm from all and thus gradually vacuolates a large space in which it is more and more free to move. Thus a moderate infection with *C. delphinia* is more destructive to its flea host than is a similar infection of *S. lactaria* to its milliped host.

After the whole parasitized space has been vacuolated and the nourishment has been exhausted, and when the walls next the lumen along with their ciliary boundary have disintegrated (Fig. 8), the parasite is free to give up its confinement and becomes free-living within the lumen of the canal. It is quite probable that the limiting wall is often violently ruptured by the increasingly active movements of the animal.

After the parasite has liberated itself and migrated into the lumen, it becomes a sporont. This departure is entirely due to accident, the parasite in its movements finding the opening which has been made into the lumen. The mode of assimilation of the sporont undergoes little change from that of the trophozoite; instead of receiving its

nourishment from the cell, food is absorbed before it is taken up by the cell. The animal leads a more or less sluggish life even when freed, for groups are found lying close to the cilia and embedded in mucous masses (Fig. 9; see also Watson, 1916, Fig. 3). Mature live sporonts measure approximately 110μ by 60μ .

After the cell-group is rid of its exhausting burden, it is practically destroyed; there is little protoplasm left and no nuclei to recover from the shock and with which to reconstruct the shattered group. Certain small barren areas are sometimes seen in cross-sections of the epithelium, and these structureless regions may be caused by destruction due to parasites. There are two other possibilities, however, concerning the vacated cells: The surrounding unaffected cells may gradually enlarge and close entirely over the space, or regeneration of the destroyed tissue may take place.

In instances of maximum infection noted, the effect of parasitism upon the host is undoubtedly deleterious. When twenty or more cells are completely destroyed by each parasite of a hundred or more developing at the same time, the absorptive capacity of the intestine is greatly diminished. I was, however, unable to prophesy from the action of live fleas which would be the more prolific in parasites, tho this had been done in the case of some hosts parasitized with enormous numbers of gregarines, or nematodes, or both (e. g., various grasshoppers and *Diabrotica vittata*, the cucumber beetle).

The parasite does not attain its maximum growth within the epithelium but grows considerably after becoming free (contrast Figs. 8 and 10). During its sporont life it becomes attached to another of its kind and ultimately the two conjoin to form a cyst. This process has already been described for the species in question (Watson, 1916:131).

Three species were described by the writer in 1916 as *Frenzelina delphinia*, *F. olivia* and *F. nigrofusca* from beach fleas, fiddler crabs and littoral spider crabs. I find in a footnote in an article by Léger and Duboscq (1911) that the genus name *Frenzelina* Léger and Duboscq (1907) is preoccupied by a rhizopod, *Frenzelina* Penard 1902; and that the genus name for the gregarine becomes *Cephaloidophora*, given by Mawrodiadi in 1908. Therefore my three species become, respectively, *Cephaloidophora delphinia*, *C. olivia* and *C. nigrofusca*.

SUMMARY

Consecutive stages in the growth of *Cephaloidophora delphinia* (Watson) are shown.

This species does not possess an epimerite.

Development is intracellular.

Many cells contribute to the nourishment of the parasite, all of which are ultimately destroyed.

No noticeable hypertrophy of either nucleus or vegetative protoplasm of the host cells is indicated.

Large infections are deleterious to the host.

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EXPLANATION OF PLATE

The reference line is 0.03 mm. long.

Fig. 1.—Alimentary tract of *Talorchestia longicornis* (Say), from preserved material, *h c*, hepatic caeca; *m i*, mid-intestine; *e c*, excretory caeca; *p* proctodeum.

Fig. 2.—Early stage in growth of trophozoite, septumless, host cell-nucleus already affected.

Fig. 3.—Later stage, similar to last, contiguous cells being vacuolated.

Fig. 4.—Two cross-sections of young trophozoites, showing marked influence upon the epithelium.

Fig. 5.—Half-grown trophozoite with septum formed.

Fig. 6.—Parasites in three situations within host-tissue; (*a*) cross-section, position most favorable for absorbing nourishment from many cells; (*b*) oblique position, with marked destruction of tissue; (*c*) longitudinal section of nearly full-grown trophozoite.

Fig. 7.—Third position of parasite, parallel to long axis of the cells. Cell wall toward lumen weakened.

Fig. 8.—Cell wall broken preparatory to trophozoite migration.

Fig. 9.—Sporonts in lumen of upper proctodeum.

Fig. 10.—Mature associated sporonts, free in upper portion of proctodeum. Glycerine mount.

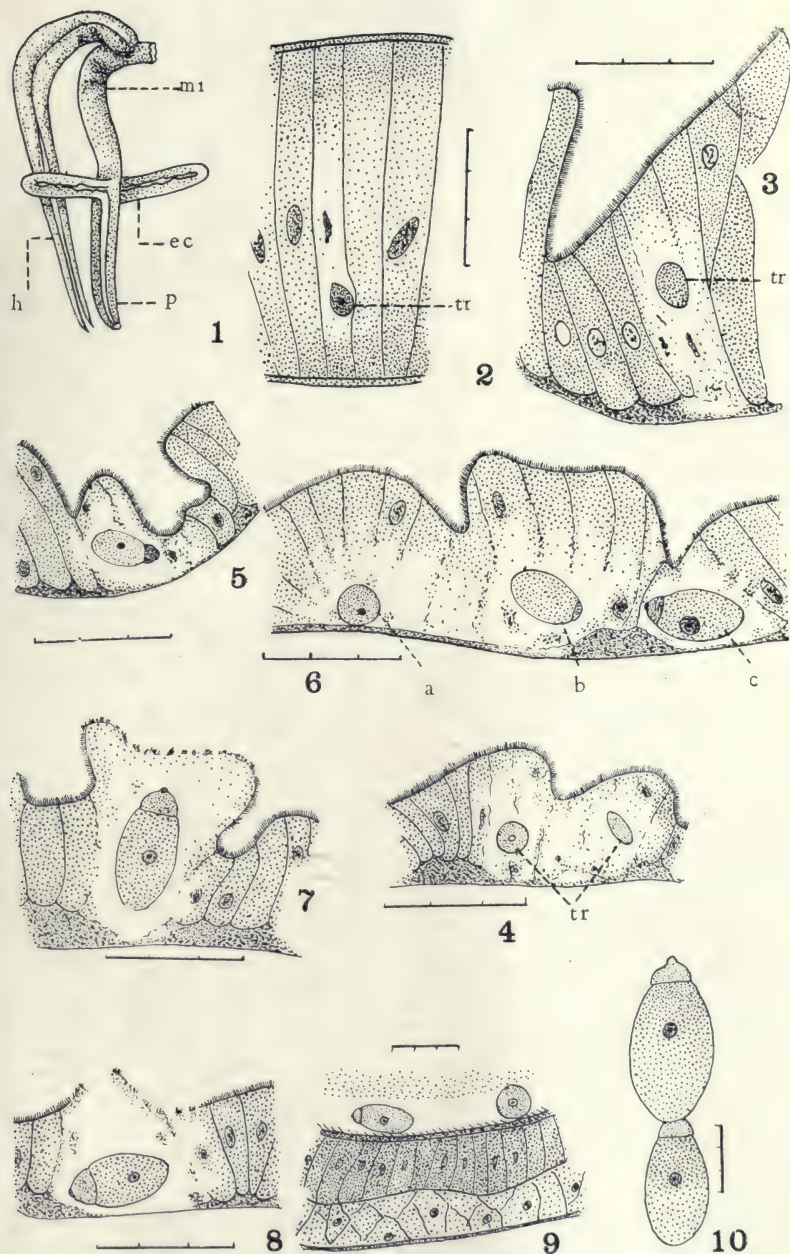


PLATE IV



ON THE LIFE CYCLE OF THE FOWL CESTODE,
DAVAINEA CESTICILLUS (MOLIN)*

JAMES E. ACKERT
(Preliminary Communication)

Little is known of the means by which tapeworms are transmitted to fowls. Of the six species recorded from chickens in the United States, the life histories of but two have been demonstrated experimentally. *Davainea proglottina* (Davaine), rare in this country, may be transmitted by the slug, *Limax cinereus* Lister, according to Grassi and Rovelli (1889:372; 1892:86), and *Chōanotaenia infundibuliformis* (Goeze) may have the house fly, *Musca domestica* Linn., (Gutberlet, 1916:235) as its intermediate host.

Recently, the writer has demonstrated experimentally that *Musca domestica* may transmit to chickens another tapeworm which appears to be *Davainea cesticillus* (Molin 1858) Blanchard 1891. The fowls used in the experiment were hatched in an incubator and placed at once in a fly-proof field cage which, with its floor and 18-inch walls of cement, is so constructed as to be worm-proof also. Examinations of control chickens every few weeks for four years have not yielded a single parasitic worm. In this field cage, the fowls were given food free from animal tissues, with the exception of some fresh beef and the experimental feedings.

House flies taken from nature were placed in small lantern globe cages and given living onchospheres from the fowl cestode, *D. cesticillus*. Onchospheres and portions of teased, gravid proglottids in a small drop of water were eagerly taken by the flies. The latter were then kept alive as long as possible to afford time for the development of larval tapeworms (cysticercoids) in the bodies of the flies. By giving to these caged flies small amounts of whole, sweet milk, fairly large numbers of them were kept alive for two or three weeks after feeding the onchospheres. As soon as the flies died, they were either preserved for sectioning or given to young chickens reared in the fly-proof field cage. In this way several hundred house flies were fed, a few at a time, to twelve chickens.

From these fowls, nine cestodes were taken, two from chick No. 165 being sexually mature. Control chickens from the same broods kept with the experimental ones in no case yielded a parasitic worm.

* Contribution No. 24 from the Department of Zoology, Kansas State Agricultural College. Aid of Adams Fund.

Chick No. 165 was given a total of 245 house flies from August 25 to September 11, and on October 13 the examination revealed two cestodes in the small intestine both of which possessed gravid proglottids. They are described in the following.

DIAGNOSIS

Length, 79 to 90 mm. Maximum width, 1.6 to 2.3 mm. Scolex (Fig. 1) cylindrical, 0.5 to 0.6 mm. wide and 0.3 to 0.4 mm. long. Suckers unarmed, about 0.1 mm. in diameter. Rostellum broad and hemispherical, 0.29 mm. wide, with approximately 200 very unstable hooks. Hooks (Fig. 2), 8 to 9μ long, with long ventral and short dorsal root. Neck very short, followed by proglottids equal to or greater in width than the scolex. Anterior proglottids, 3 to 6 times as broad as long; following ones increasing in size until length exceeds width; borders (Fig. 3) overlapping. Genital pores irregularly alternate, one in each proglottid somewhat in front of middle of lateral margin in young proglottids and nearer middle in older ones. Vagina and cirrus pouch (*c*) on dorsal side of two excretory canals and nerve.

Male reproductive organs: Testes (*t*), 20 in number in posterior portion of proglottid. Vas deferens (*v d*) coiled before entering base of cirrus pouch, also coiled within latter. Cirrus pouch ellipsoidal, 139 to 164μ long by 65 to 82μ wide. Cirrus when protracted, 131μ long and 13μ in diameter, armed with minute spines, and with bulbous enlargement 21μ in diameter at base becoming continuous with cirrus pouch.

Female reproductive organs: Vagina enlarged near median line into small seminal receptacle. Ovary (*o*) in middle field in front of testes. Yolk gland and shell gland posterior to ovary, ventral and dorsal, respectively. Uterus at first in front of ovary; gradually increasing in size, finally extending laterally to excretory canals and occupying most of proglottid; in oldest proglottids dividing into compartments or capsules each containing a single egg. Embryo (Fig. 4), 35 by 31μ in diameter, with very thin membrane closely adherent to its surface; embryo further enveloped by thicker, smooth membrane, oval in shape, 42 by 36μ in diameter; latter surrounded by thin, wrinkled membrane approximately 66 by 61μ in diameter; embryo and membranes finally enclosed in capsule of outer and inner layer.

The characteristic scolex with its broad, flat rostellum, the anterior proglottids as wide as the scolex, and the eggs in individual capsules in the posterior proglottids, together with the other diagnostic points

ACKERT—LIFE CYCLE OF *DAVAINEA CESTICILLUS*

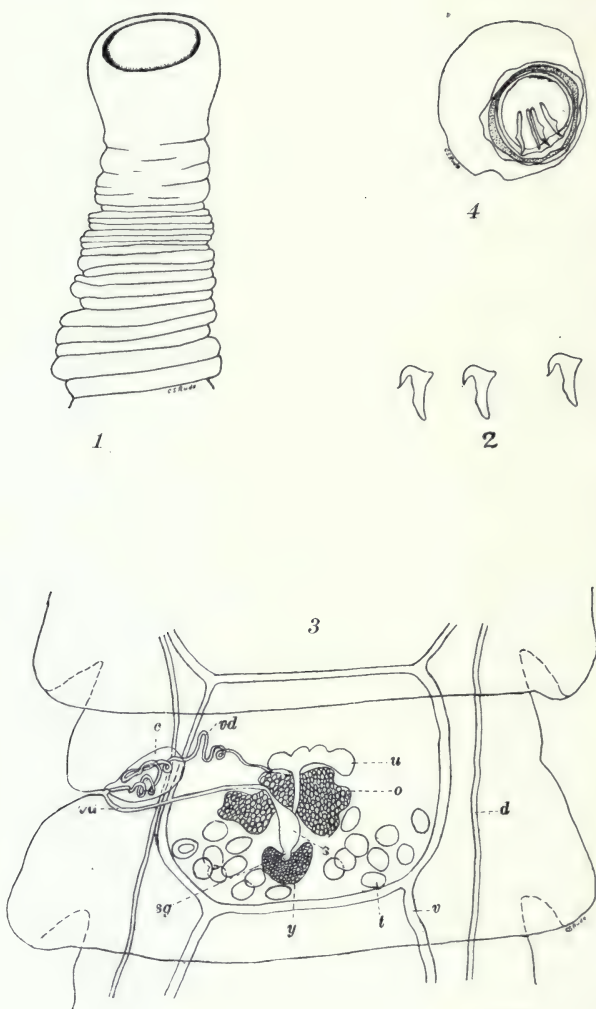


PLATE V

appear to leave no doubt as to the identity of these worms. As the house flies constituted the only animal tissues (except fresh beef) given to the experimental chickens, the writer concludes that *Musca domestica* may transmit *Davainea cesticillus* to fowls.

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EXPLANATION OF PLATE

- Fig. 1.—Scolex showing broad, flat rostellum and anterior proglottids. $\times 62$.
 Fig. 2.—Rostellar hooks. $\times 480$.
 Fig. 3.—Mature proglottid. Certain details were added from studies of transverse sections. $\times 103$.
 Fig. 4.—Embryo. $\times 668$.

The drawings were made with the aid of a camera lucida.

ABBREVIATIONS

<i>c</i> , cirrus pouch	<i>d</i> , dorsal excretory canal
<i>o</i> , ovary	<i>s g</i> , shell gland
<i>s</i> , seminal receptacle	<i>t</i> , testes
<i>u</i> , uterus	<i>va</i> , vagina
<i>vd</i> , vas deferens	<i>v</i> , ventral excretory canal
	<i>y</i> , yolk gland

REVIEWS AND NOTES

Under the title of "The Malaria Problem in Peace and War," Dr. Frederick L. Hoffman has published a valuable study of (1) modern methods for the eradication of malaria and their results, and (2) the relations of this disease to war. It is an exceedingly comprehensive presentation of materials from official sources, so condensed and well arranged as to be generally useful.

The 1917 annual report of the medical department in the United Fruit Company puts malaria as the most prevalent disease with one-third of all their cases; in 28,985 cases, however, they had only 54 deaths. Second in importance was hookworm; the records seem to show a distinct gradual decrease in the number of these cases in recent years. Amebic dysentery and a type of flagellate dysentery are also mentioned prominently in the report. *Clonorchis sinensis* was recorded from a Chinese laborer in Cuba, the first record for that region.

The Division of Biology of the California State Board of Health has been designated the Division of Parasitology. The program of this Division includes not only practical work on the hookworm in the mines of California, and on the parasites among the oriental and Mexican laborers of the state, but also a program of general research work in the field of parasitology. The division of parasitology is planning to build up a library and will be glad to receive publications along parasitological lines. The publication of special bulletins is provided for in the plan. Dr. C. A. Kofoid will be consulting parasitologist and Dr. W. W. Cort, consulting helminthologist.

It is with deep regret that the workers in parasitology have learned of the death, on February 16, at the age of 64, of Dr. F. M. Sandwith, C.M.G. Dr. Sandwith was well known for studies on tropical diseases, and was connected with the London School of Tropical Medicine.

Professor W. A. Riley, of the editorial board of THE JOURNAL, has been appointed professor of entomology and chief of the division of entomology and economic zoology at the University of Minnesota. He should be addressed at University Farm, St. Paul, Minnesota. By an error, Professor Riley's initials were misprinted in the March number of THE JOURNAL, on page 139.

In view of the evident need for army work of men, trained in the special field, a group in Washington, D. C., has been engaged in the study of disease-transmitting insects and methods for their control. Dr. W. Dwight Pierce has taken the leadership of the work which includes not only meetings for study and discussion, but correspondence with field men as well.

EDITOR'S NOTE

An unfortunate error, for which the author was not responsible, was made in the paper by M. W. Kamm in the last number of THE JOURNAL; the paragraph

GREGARINA DIABROTICA nov. spec. (Figs. 5 and 6)

Host: *Diabrotica vittata* Fabr. (Chrysomelidae).

Location: Urbana, Illinois, June, 1917.

Habitat: Intestine.

was printed at the bottom of page 162 instead of directly after the table at the bottom of 161, where it properly belongs.

The actual dates of issue of Volume IV of THE JOURNAL were as follows:

No. 1, October 16, 1917.

No. 3, April 8, 1918.

No. 2, January 19, 1918.

No. 4, September 14, 1918.

The Journal of Parasitology

Volume 5

DECEMBER, 1918

Number 2

NOTES AND EXPERIMENTS ON *SARCOCYSTIS* *TENELLA* RAILLIET

II. SEASONAL INFECTION

JOHN W. SCOTT

In a former paper (1915) the writer stated some evidence favoring Darling's suggestion that Sarcosporidia are merely aberrant varieties of the Neosporidia of certain invertebrates. At the time it was mentioned that experiments were in progress to test the question whether this hypothesis was correct or not. Results so far have shown that the control lambs were not entirely free from infection, so a report on these experiments will be deferred until a later paper. Certain definite data have, however, been obtained with reference to the time of year and other conditions under which infection does and does not occur. The present paper will discuss such data. By using more rigid methods of examination it has been found that apparently 100 per cent. of all adult range sheep are infected with sarcocysts. In the work reported here the lambs examined for sarcocysts were raised under various conditions. Except as otherwise stated the experimental lambs were kept in a dry lot from the time of birth; were supplied with city water which comes from deep springs, and were fed baled native hay kept over from the previous season. Those lambs over which there was no control are referred to as range lambs. By a reference to Table 1 it will be noted that in all groups where grazing was involved 100 per cent. of the lambs became infected. This was true whether the lambs were grazed on the range, in dry or wet pasture, or were fed grass, or flowers, taken from the wet pasture (Groups 1, 2, 4, 5, 6). It is also true that seven out of nine of the control lambs became infected (Group 3). Group 8 was treated as control lambs except that each of the eight lambs used was fed twice weekly a different kind of insect. Six out of eight of these lambs became infected. However, two of these lambs had a very heavy infection, in fact, heavier than any of the groups listed except Group 1, which included the range lambs. A full account and a discussion of the significance of these results will be given at another time. They are mentioned here to show the

different conditions under which the lambs were raised from which some of the important data for this paper was derived, and to indicate that one probably should not expect entire uniformity with respect to the time or degree of infection.

TABLE 1.—TO SHOW INFECTION UNDER DIFFERENT CONDITIONS

Groups	Number in Group	Treatment	Number Infected	Per Cent. Infected
1	5	On range until killed.....	5	100
2	6	Wet pasture, July 15 to September 23.....	6	100
3	9	Control. Kept in dry lot; no green food.....	7	77.7
4	6	Dry lot; grazed twice weekly in lot around pond.....	6	100
5	2	Dry lot; grazed twice weekly in dry grassy pen near pond.....	2	100
6	2	Dry lot; fed grass and flowers from wet pasture twice weekly.....	2	100
7	1	Dry lot; watered twice weekly from pond of Group 4.....	1	100
8	8	Dry lot; each fed a different kind of insect twice weekly.....	6	75

Group 1 consisted of early spring lambs; other groups were all May lambs. In all lots ewes ran with their lambs.

Of the lambs grouped in Table 1, three of the range lambs were killed July 13, and the other two six weeks later on August 24. Of the experimental lambs, six were killed October 27; six on November 3; six on November 10; five on November 17; six on the following January 13, and five about two months later, on March 16. In each of these lambs the tip of the heart muscle was saved, fixed in absolute alcohol, embedded, cut in serial sections 16μ in thickness, and stained with Delafield's hematoxylin and eosin. This treatment brings out the sarcocyst in sharp contrast to the muscle tissue in all except the earlier, or Bertram's stages, to find which one needs to use a high power and mechanical stage to avoid overlooking them among the muscle nuclei. The work has involved the preparation of several hundred slides. Altho infection is generalized, the tip of the heart was used for examination, since it is easily localized and conditions are uniform.

The sarcocysts vary considerably in shape, dependent chiefly upon location in the body, and partly upon arrangement of the muscle fibers and connective tissue. For example, in the diaphragm the sarcocysts may be much elongated, and may approximate a spindle shaped form. The typical sarcocyst in the heart, however, is circular in cross section and in longitudinal section is approximately the form of an ellipse. In order to readily obtain a measure of volume it was decided to square the mean diameter and multiply this result by the length of the sarcocyst. This product gives the volume of a rectangular solid just large enough to enclose the sarcocyst measured, and generally is very nearly proportional to the volume. Some sarcocysts were too irregular in shape to obtain an accurate measure of volume, and these were excluded from the calculations. In early stages the length is usually

several times the diameter, but later the diameter increases more rapidly than the length. All measurements were made by means of an eyepiece micrometer, and calculated in micra.

In working over the material to determine whether infection had occurred under the various conditions enumerated above, it was observed that the older the lamb the larger was the sarcocyst; this was to be expected. It was also noticed that in those lambs killed in January and March small sarcocysts appeared to be very scarce or entirely wanting. This seemed to indicate that infection did not occur during the winter and suggested a number of problems. Is infection continuous or discontinuous? Does infection occur only in young lambs? That is, does a range lamb become infected for all time while it is a lamb? Or, is infection seasonal, and does it recur year after year? In an attempt to answer these questions it was decided to take careful measurements of all sarcocysts found in these experimental lambs, and in other lambs and old ewes killed at various seasons of the year.

TABLE 2.—TO SHOW THAT THE AVERAGE SIZE OF THE PARASITES INCREASES WITH THE AGE OF THE LAMB

Group	Date Killed	Lambs Examined	Sarcocysts Measured	Mean Diameter	Mean Length	Mean Volume \times Mean D ² \times Mean L.
1	July 13	3	29	29.7	101.7	89,708
2	Aug. 24	2	20	29.6	119.5	104,701
3	Oct. 27-Nov. 17	19	109	30.4	106.3	98,238
4	Jan. 13	6	51	34.5	120.4	143,306
5	Mar. 16	5	39	41.9	110.6	194,170
6	Oct. 27-Nov. 17	3	17	28.3	101.2	81,050
7	Jan. 13	3	25	34.0	125.5	145,078
8	Mar. 16	1	9	48.8	100.3	238,858

Measurements are given in micra. Group numbers do not correspond to groups of Table 1.

A reference to Table 2, shows that the average size of the parasite increases with the age of the lamb. A brief explanation will make this clear. The first column gives group numbers; the second, the dates when the groups were killed; the third column gives the number of lambs examined, and the fourth the number of sarcocysts measured in each group; in the fifth and sixth columns are found the mean diameter and mean length of each group of sarcocysts; in the seventh column is found the mean volume of each group of sarcocysts given in cubic micra; this volume was obtained by squaring the mean diameter and multiplying by the mean length. Groups 1 and 2 were early spring range lambs, the exact age of which is not known; they were, however, as large in August as the experimental lambs were in October, and were probably born in March or early April. Comparing these two groups, killed just six weeks apart, we find the mean volume of the sarcocysts has increased from 89,708 cubic micra on July 13 to 104,701 cubic micra on August 24. Groups 3, 4 and 5 were late April and May lambs; these were all kept in a dry lot until July 15, when

there was begun the experimental work noted above. Groups 4 and 5 were killed at about nine week intervals after Group 3. Here too we find a gradual increase in the mean volume of the parasites. Groups 6, 7 and 8 represent infected control lambs, the data from which are included under Groups 3, 4 and 5, respectively; the data for the control lambs are given separately to show that the same general fact holds in lambs receiving identical treatment and killed at different times.

The fact is also brought out in Table 2 that the mean length, or mean diameter, of a group of sarcocysts is not necessarily proportional to the age of the lamb. In other words, the mean diameter, or mean length, is a variable dependent upon the nature of the tissue in which the sarcocyst is located. The amount of growth increases with the age of the lamb, but the direction of growth is a function of the tissue and depends upon other factors than age. There is also evidence to show that the rate of growth is faster in some lambs than in others; and that sarcocysts are retarded somewhat in growth if they chance to find lodgment in a location unfavorable for nutrition. Since the direction of growth may take place in three directions and since the sarcocyst reproduces by spore formation, it is to be expected, other factors being equal, that the volume of a sarcocyst will increase in a geometrical ratio with arithmetical increments of time. Such data as are available strongly indicate that this conclusion is correct.

That the mean volume of the sarcocysts would increase with the age of the lamb was to be expected. One would anticipate this result whether infection is a continuous or a discontinuous process. Now if infection is continuous, in lambs killed at successive intervals, one may expect the ratio of the volume of the smallest sarcocyst to the volume of the largest sarcocyst to increase gradually with the age of the lamb. But if infection is discontinuous, one may expect this ratio to increase until after the infective period is over and then gradually decrease, until after the time arrives for reinfection.

TABLE 3.—TO SHOW THAT INFECTION IS DISCONTINUOUS

Group	Date	Number Lambs Used	Sarco- cysts Measured	Mean Volume of Parasites	Ratio of Volume Smallest : Largest	Ratio of Volume Smallest : Mean
1	July 13	3	29	89,708	1 : 70.18	1 : 11.95
2	Aug. 24	2	20	104,701	1 : 75.87	1 : 18.21
3	Oct. 27-Nov. 17	19	109	98,238	1 : 109.76	1 : 18.60
4	Jan. 13	6	51	143,906	1 : 9.71	1 : 2.43
5	Mar. 16	5	39	194,170	1 : 8.11	1 : 3.77
6	Oct. 27-Nov. 17	3	17	81,050	1 : 21.08	1 : 9.42
7	Jan. 13	3	25	145,078	1 : 3.09	1 : 1.91
8	Mar. 16	1	9	238,858	1 : 6.00	1 : 3.42

Same groups compared as in Table 2. Note that while the mean volume of the sarcocyst increases with the age of the lamb, after a considerable period the ratio of the smallest to the largest parasite decreases instead of continuing to increase.

A study of Table 3 shows that this in general is what one finds. The ratio of the smallest to the largest of twenty-nine sarcocysts killed on July 13 was 1 to 70.18. By August 24, of twenty sarcocysts measured, the ratio had increased to 1 to 75.87, thus indicating continuous infection. These ratios probably do not give the best sort of a comparison, for if one leaves out of consideration the largest parasite for each date, the ratios become, on July 13, 1 to 35.18 and on August 24, 1 to 57.79. It should be mentioned that the smallest parasites found for these dates had about the same volume. Or, if one takes the ratio of the smallest parasite to the mean volume, it becomes for July 13, 1 to 11.95, and for August 24, 1 to 18.21. In any case both the ratios and the mean volume increase, while the size of the smallest parasite found does not increase. Consequently, infection has been continuous throughout this period.

Now in the experimental lambs, killed October 27 to November 17, the smallest sarcocysts found were about the same size as the smallest found during the summer, but the ratio of the smallest to the largest at this date is 1 to 109.76, and the ratio of the smallest to the mean is 1 to 18.60. Comparing these smallest stages found with the stages figured by Erdmann (1910), it is probable that they represent an age 5 to 7 weeks after infection, and from 1 to 3 weeks after invasion of the musculature. It is probable therefore that infection occurred in these lambs up to about October 1. It is also interesting to make a comparison with the results obtained by killing lambs on January 13 and March 16. The smallest parasite found on these dates had volumes between 50,000 and 60,000 cubic micra, while the smallest parasites found during the summer and fall were only one-ninth or one-tenth as large. On January 13 the ratio of the smallest to the largest parasite was 1 to 9.71, and its ratio to the mean was 1 to 2.43. So one finds that a large decrease in the ratio has occurred in spite of a large increase in volume, and this decrease is due to the fact that small sarcocysts are no longer found. The same holds true on March 16. Here the ratio of the smallest sarcocyst to the largest is 1 to 8.11; that is, the ratio is still decreasing. The ratio of the smallest to the mean volume is 1 to 3.77. This is somewhat larger than the corresponding ratio for January 13, and is accounted for by the fact that the smallest parasite found in these lambs on March 16 had about the same volume as the smallest one found on January 13. A larger series of measurements for these dates would no doubt render more uniform results. I have proof to show that this would be true. In an old ewe killed December 28 the smallest sarcocyst had a volume of 38,304 cubic micra. Considering the rate of growth, one would expect the smallest parasite found on January 13 to have a volume between 40,000 and 50,000 cubic micra, instead of 58,000 as was the case in the few experimental lambs killed on this date. Groups 6, 7 and 8 represent the

results from the control lambs, given separately. Here too the results, to a certain extent, lack uniformity, due to the small number of parasites involved. However, in all cases *it is clear that infection has been discontinuous*, and probably there was *no infection after about the first of October*.

Another question, with reference to how early in the spring does infection begin, has not been so accurately determined. If all sarcocysts had the same rate of growth, one could measure the largest sarcocysts found in lambs at different dates during the summer, and could determine fairly accurately the approximate time when infection begins in the spring. The rate of growth, however, of a particular sarcocyst appears to depend to a considerable extent upon the amount of nutrient fluid, and pressure of the tissues, surrounding it. It is noticeable that the larger sarcocysts of a lamb of given age killed during the winter are in the looser, more vascular portions of the heart muscle. Likewise the smallest sarcocysts of such a lamb are found in the more compact and denser regions of the heart tissue. Consequently to draw conclusions from the study of a few sarcocysts will not be satisfactory, and up to this time there has been no opportunity to study a large body of controlled material taken in the spring or summer. Still, some of the results are interesting.

An old ewe, No. 226, was killed June 13. A very careful examination showed that the smallest sarcocyst found had a volume of 102,513 cubic micra. Evidently this parasite belonged to infection during a preceeding season, for one would expect to find much smaller sarcocysts if infection had begun the current spring. Again, the smallest sarcocysts found in March (Group 5, Table 3) had volumes between 53,000 and 60,000 cubic micra, and one would expect these to have a volume in June of over 100,000 if they increased in size at an average rate (see Table 3 for average rates of growth). So if one allows six weeks for the infection to appear in the muscles, reinfection had not begun (Ewe 226) on May 1. However, one must not overestimate this evidence for only twelve sarcocysts were found in the material preserved from this ewe; she had had small chance of infection recently and she had not been outside of a dry feed lot for more than twenty months. Perhaps if this ewe had been running on the range some small sarcocysts would have been found.

Two lambs, 986 and 996, one born March 26, and the other April 3, were killed June 22. No sarcocysts were found in either of these lambs. For a considerable portion of the latter part of April and during May they had access to pasture conditions. Beginning the first week in June they were kept in a dry lot until killed. If infection occurs without the necessity of any other host, one would expect to find sarcocysts in these lambs, since they were both old enough to show the infection and had been exposed to pasture conditions. Inci-

dentally, it may be mentioned that, owing to a late season, practically no insects were present up to the date when they were killed. Lamb 302, born early in June, died July 20. On June 13 this lamb had been taken from the ewe to be raised by hand, and had no contact with sheep after this date. It did not begin to eat grass to any extent until about the end of June, and if it became infected, the sarcocysts had not yet appeared in the muscles. Lamb 286, born about the middle of May, was kept in a dry lot until it died, July 28. No sarcocysts were found. It had been fed certain insects on July 17, 20, 24 and 27. Conditions for infection were not favorable, and this negative result is of no value. Lambs 284 and 294 born in May were killed August 9. They were kept in a dry lot until July 16, after which they were turned out to graze in a pasture where 100 per cent. infection had always been obtained in all lambs. These lambs, had, therefore, probably become infected, but the sarcocysts had not yet appeared in the heart muscle twenty-four days after their first exposure to favorable conditions for infection. Lambs 283 and 291, born in May, were killed September 22. These lambs were treated like 284 and 294, but were allowed to run in the pasture forty-four days longer, a total of sixty-eight days in pasture. In the material sectioned, one sarcocyst with a diameter of 15.4 and a length of 44μ was found in the heart muscle of lamb 282. So far as studied, no sarcocysts were found in lamb 291. The size of the sarcocyst indicates that this infection could not have occurred much, if any, later than August 1, assuming it takes forty days for the parasite to become established in the muscle tissue. The evidence presented above with reference to how early in the spring infection begins, is of no great value. However, it appears that infection does not begin very early in the spring, and probably at least five or six weeks must intervene between actual infection and the appearance of the sarcocysts in the muscle tissue. Altogether, *it is very clear that there is a distinctly marked seasonal infection*, and this fact is in agreement with the idea suggested in a former paper that *Sarcocystis tenella* may be primarily dependent upon some invertebrate, probably some insect host. But the solution of the question whether Sarcosporidia are aberrant forms of Neosporidia as maintained by Darling (1915) and supported by Scott (1915), or are identical with Cnidosporidia, as suggested by the observations of Piana (1896) and Galli-Valeria (1916), must depend upon other data. It is believed that certain experiments now in progress will throw light on this problem.

Since infection is hard to control, and since practically all sheep of this region are infected, the theory has been suggested that infection was possibly transmitted *in utero*. There is strong evidence against this idea. A lamb, No. 301, died shortly after birth. The mother was killed soon afterward and proved to be an old heavily infected ewe. No sarcocysts were found in the lamb. In our experiments other older

lambs, from infected mothers, have also been found free from infection. In this connection considerable work has been done. Bertram (1892) examined the embryos of sheep, swine and cattle, but found no sarcocysts in any of them.

Bergmann (1913) found sarcocysts in 8 per cent. of lambs 6 to 10 weeks old, but he found that 20 per cent. of lambs just a little older (3 months) were infected. M'Gowan (1914), who believes in congenital infection, examined in 1913 "a large number of embryos from heavily infected mothers," but nevertheless found no infection; he also examined a number of lambs at various ages and the earliest age at which he found infection was 3 months. In the following year he made a more critical examination, by means of serial sections, of lambs 2, 16, 18, 22, 27 and 29 days old, but found no sarcocysts. All the evidence is against the theory that infection occurs *in utero*. The gestation period of the sheep is nearly five months, and considering the cotyledonous type of placenta at least ten or twelve weeks of the period should be favorable for infection. If six weeks is added to the period favorable for infection, since no lambs under 6 weeks have been found infected, sixteen or eighteen weeks appears as probably the shortest time from infection until the appearance of the parasites in the muscles. But in the case of *S. muris*, according to Smith and Nègre, the time from infection to appearance of the parasites in the muscles may be as short as forty-five days, and there is no apparent reason why the time for *S. tenella* should be longer. Now Bergmann is the only investigator who has found sarcocysts in the muscles of lambs at an age approximating this short period, namely in lambs 6 to 10 weeks old. It then appears that infection after birth agrees with all facts yet brought forward, and all the evidence is opposed to the idea of infection before birth.

One may now consider the question, does infection occur only in young lambs, or does it recur in successive seasons? The proof is apparently conclusive that the latter alternative is correct. In the old ewes one finds small sarcocysts, and in addition large ones that are immensely larger than any of those found in lambs during the first year. This proves that infection extends over more than one year; that is, infection may and probably does recur in successive seasons as long as the sheep lives. An inspection of Table 4 will help to make this matter clear.

In the case of Ewe 226, killed about the middle of June, the volumes of the nine parasites given apparently fall in three or four groups, and so probably represent infection in three or four different seasons or years. Parasites 1 to 4 no doubt belong to infection one year back; parasites 6 to 8 are probably in their second or possibly third year; and parasite 9 is separated widely from number 8, and is

seemingly of older growth, perhaps about 3 or 4 years old. Since the largest sarcocysts found in lambs killed in March (Group 6, Table 3) were less than 600,000 cubic micra, sarcocyst 5 may be only about one year old, but it probably belongs to the second season preceding. However, one cannot determine with certainty the age by the size of

TABLE 4.—TO ILLUSTRATE THE VOLUMES OF SARCOCYSTS IN OLD EWES AND TO SHOW THAT INFECTION EXTENDS THRU MORE HAN ONE SEASON

Sarcocyst Number	Ewe No. 226 Killed June 13	Ewe No. 4 Killed October 24	Ewe No. 717; Killed December 28		
	Volume	Volume	Volume	Sarcocyst Number	Volume
1	102,513	4,702	27,684	51	298,144
2	182,023	5,712	30,178	52	299,625
3	185,526	6,701	38,304	53	309,217
4	185,856	6,751	42,592	54	327,703
5	644,335	9,820	45,900	55	332,750
6	1,018,484	18,817	57,584	56	332,750
7	1,354,896	56,453	61,327	57	340,139
8	1,741,610	90,626	64,420	58	383,029
9	2,861,932	93,828	71,874	59	404,624
10	96,341	89,975	60	412,280
11	126,737	92,248	61	417,404
12	133,816	119,700	62	439,357
13	134,853	119,700	63	446,880
14	136,294	119,700	64	450,410
15	137,773	122,998	65	453,508
16	146,232	122,998	66	484,183
17	147,581	124,104	67	519,090
18	156,119	125,390	68	545,177
19	157,767	125,390	69	578,985
20	169,458	130,842	70	622,908
21	172,497	131,068	71	665,000
22	180,495	131,769	72	704,365
23	234,890	137,998	73	709,582
24	249,356	143,748	74	776,354
25	275,272	143,748	75	795,659
26	283,947	144,472	76	851,840
27	297,360	144,812	77	862,488
28	311,364	146,143	78	863,755
29	325,075	153,863	79	875,844
30	338,875	172,327	80	1,042,720
31	372,956	172,491	81	1,120,234
32	377,018	172,497	82	1,225,017
33	429,143	179,685	83	1,245,816
34	453,955	179,902	84	1,333,312
35	456,744	180,241	85	1,596,000
36	543,250	183,144	86	1,697,376
37	561,009	184,497	87	1,770,602
38	732,674	189,747	88	1,788,864
39	754,252	190,812	89	1,862,400
40	765,215	193,197	90	1,931,160
41	766,656	196,196	91	2,793,100
42	766,656	204,441	92	2,863,436
43	1,072,599	206,841	93	3,194,400
44	1,384,825	212,960	94	3,194,400
45	1,397,439	225,142	95	4,599,936
46	1,893,959	245,326	96	5,398,536
47	1,992,623	250,360	97	6,673,340
48	268,329	98	6,965,977
49	281,107	99	9,171,411
50	281,107	100	16,959,640

the parasite, after the first year, on account of the different rates of growth of different sarcocysts. At the end of the second year the largest parasites of that year are overlapping in size the smallest due to infection the first year. By accumulating a vast amount of data one could possibly determine the probable age of each sarcocyst. Ewe 226 was more than 3 years old, but her exact age is not known.

From range Ewe 4, killed October 24, there was obtained a larger series of sarcocysts. A comparison of the volumes of the forty-seven sarcocysts measured with the volumes of the sarcocysts of the experimental lambs shows that the parasites of this ewe probably owe their origin to infection during three different seasons. Sarcocysts 1 to 22, and possibly 23 and 24, belong to the summer just past; sarcocysts 25 to 42 are probably in their second year, while 44 to 47, and probably 43, are in their third year. In any event the measurements show that infection has taken place in more than one season. No information was obtainable in regard to the age of this ewe. In reference to this group of sarcocysts another fact is of importance. The smallest sarcocysts found (Table 4) are about the same size as the smallest sarcocysts found in the experimental lambs killed October 27 to November 17. That is, infection occurs about as late in the season in old ewes as it does in lambs.

A still larger series of parasites was measured from Ewe 717, which was killed on December 28 at the age of 3 years and 9 months. This ewe had, therefore, been exposed to four seasons of infection. By a comparison with the results obtained with the lambs killed at different dates, the average size of the parasites of the fourth, that is, the immediately preceding summer, should be about 132,000 cubic micra. By a comparison with the largest sarcocysts found in lambs killed January 13, parasites 1 to 45 (Table 4) all belong to this fourth season. By a similar comparison, parasites 46 to 79, inclusive, with volumes varying from 245,326 to 875,834 cubic micra, should probably be assigned to the third, or second, preceding season. Sarcocysts 80 to 94, with volumes varying from 1,042,720 to 3,194,400 cubic micra, would seem to belong to the second, or third, preceding summer, and parasites 95 to 100, inclusive, probably belong to the first, or fourth, preceding season of infection. Here too it is evident that infection extended through more than one season. A study of Table 4 brings out another point of interest. If ewe 717 was infected during four different seasons as it had a chance to be, then there could be four groups of sarcocysts with approximately the same number in each group. But if these observations are based upon adequate data and the reasoning has been correct, it appears that the older the group, the smaller the number of sarcocysts in that group. This has been found to be true in other cases besides the three ewes given, and may be accounted for in one of three ways. Either the sarcocysts gradually disappear by disintegration, as they grow older; break up into smaller sarcocysts; or some of the older sarcocysts never reach more than a moderate volume. I have no proof for or against the first alternative, there is some evidence against the second, and certain observations indicate the third view is correct. After examining many hundreds

of sarcocysts I cannot say that I have found any evidence that sarcocysts break up by disintegration. If older sarcocysts break up, thus setting free spores which wander out and become seats of new infection, this procedure in the light of facts brought out in this paper, must be an annual periodic occurrence. Aside from the lack of plausability, the proportion of small to larger sarcocysts is not compatible with such a theory. While occasionally a sarcocyst shows a stricture on one side or at one end, I have never found such a stricture approach complete separation, nor does one find sarcocysts in pairs or in fours as one would expect if such fragmentation or division of the sarcocyst did occur. Fantham and Porter, referring to their figure 55 which represents a dozen or more young pansporoblasts, state that "at this stage pansporoblasts (sometimes called sporonts) may wander out and start new infection." Even if this is true, all sarcocysts pass far beyond this stage before the second summer approaches, and another explanation will need to be given for seasonal infection. Finally, in support of the third view it has been shown that in lambs of known age the size of the sarcocyst bears a close relation to the nature of the tissue surrounding it. This is true for the first season of infection, and it would seem reasonable to believe it holds true for later stages of growth. In this way one can account for the apparent gradual decrease in the number of parasites in the older groups. Another fact supporting this view is that in some old ewes the number of sarcocysts seemingly derived from the last preceding season of infection appears rather large as compared with infection in lambs that have passed thru only one summer. In any event, it is evident that *ewes become infected year after year in successive seasons.*

TABLE 5.—TO SHOW DIMENSIONS OF SOME OF THE SMALLEST SARCOCYSTS MEASURED

Number of Lamb or Ewe	Date Killed	Diameter, Micra	Length, Micra
Range lamb 8.....	July 13	11.0	57.2
Range lamb 5.....	Aug. 24	13.2	33.0
Exp. lamb 283.....	Sept. 22	15.4	44.0
Range ewe 4.....	Oct. 24	6.6	107.9
Range ewe 4.....	Oct. 24	8.8	61.6
Range ewe 4.....	Oct. 24	9.3	78.0
Exp. lamb 280.....	Nov. 3	9.4	68.0
Exp. lamb 263.....	Nov. 10	11.0	44.0

Perhaps a mention should be made of the size of some of the smallest sarcocysts found. The dimensions given in Table 5 above will serve as a basis of comparison with the findings of other investigators. The smallest sarcocyst of *S. tenella* observed by Bertram was 6μ wide and 47μ long. This is smaller than any of those given in the table, but all of those given were apparently in the so-called Bertram stage. Crawley claims to have found a cyst consisting of a single

dividing sporoblast, and another which contained eight sporoblasts, both located intracellularly. This apparently proves that the elements in a sarcocyst undergo schizogonous multiplication. Fantham and Porter have seen all stages in this division which occurs by longitudinal fission, and they describe the growth and extension of a Sarcosporidian in a vertebrate host as follows: "Each spore contains an amoebula which finds its way into a muscle. The amoebula grows and its nucleus divides, thus becoming an elongate, multinucleate mass. Around each nucleus the protoplasm segregates, and a number of young pansporoblasts are formed. At this stage pansporoblasts (sometimes called sporonts) may wander out and start new infections (Fig. 55). Later, partitions or septa are formed between the pansporoblasts. Several spores are ultimately found in each chamber, having been formed from the pansporoblast." Judging from this description, and their figure 55, the stage at which migration may occur is the so-called Bertram's stage of various writers. The sarcocysts listed in Table 5 were all approximately in this stage, tho two or three showed the protoplasm had not yet become definitely segregated around the individual nuclei. From the data available from all sources it is probable that these parasites in Table 5 represent stages from six to nine weeks after infection.

DISCUSSION AND SUMMARY

Reference has already been made to the report of M'Gowan (1914) who wrote this lengthy paper with the purpose of showing that the disease "scrapie" is associated with and probably is caused by Sarcosporidiosis. Tho a careful reading of his paper leaves one unconvinced, this thesis leads him to postulate a partial theory in reference to the life history of *S. tenella*. As stated above, a part of this theory involves infection *in utero*, and I have presented evidence to show that this is certainly not the usual method of infection. Aside from the data which demonstrate recurring seasonal infection, the youngest age at which *S. tenella* has been found in lamb muscle is amply sufficient to account for infection after birth. No one has found sarcocysts present in lambs under 3 months old, except Bergmann, who found them in a small percentage of lambs that were somewhere between 6 and 10 weeks old. One does not know just how soon after infection *S. tenella* may appear in the muscles, but it is known (Smith, Nègre, Erdmann, Crawley) that *S. muris* appears in the muscles 40 to 50 days after infection, and there is no reason why *S. tenella* should require a longer period. Many facts are opposed to the idea of infection *in utero*, and not a single fact has been adduced incompatible with the theory that infection occurs after birth. In this connection it may be well to examine the experiment that M'Gowan gives as a crucial test.

In April and May (1913) "four lambs, from four scrapie sheep, were obtained *as soon as they were born*, before the mothers had even licked them. They were removed at first to a large byre where no sheep had ever been before, and later to a field where a similar condition prevailed. No sheep were ever allowed near them. They were looked after by an attendant whose duties did not bring him in contact with other sheep. They were brought up on cow's milk until they were old enough to live entirely on grass. When they were about one month old, living keds from scrapie sheep were applied to two of them, and these two were kept apart from the others. No further step was taken until January, 1914, in order that if sarcocysts did develop there would be no doubt of their actual presence. Then pieces of muscle were examined from the gluteal region of all four, *and in all four fully-developed sarcosporidial cysts were found in as large numbers as in lambs from scrapie mothers and of the same age brought up under natural conditions.*" . . . "and from the experiment it would appear that no conclusion could be drawn from it other than that the parasite is passed on by congenital infection of the lamb from its mother."

This conclusion might be justified if the experiment did not admit of other explanations. If Darling's insect theory of infection is correct, one would expect just as heavy infection in these lambs as in lambs which ran in fields with their mothers. Again, if infective spores are set free in the feces, as is true of *S. muris*, the possibility of infection in this manner is not entirely excluded. For, some one *brought the living keds from scrapie sheep*, and evidently here was a chance for infection by contamination. While this experiment shows that sarcosporidial infection takes place independent of the sheep tick, it affords no evidence that cannot be explained as well, or better, by theories of infection after birth.

Fantham and Porter are quoted above with reference to an early stage in the development of a sarcocyst at which the pansporoblasts may wander out and start new infections. Granted this is a common method of multiplication, it will not serve to account for seasonal reinfection, for the sarcocysts have passed beyond the pansporoblast stage before the second season arrives. It is hardly probable, that this is the usual method of reinfection, since one would not expect such an internal method of multiplication to cease on the approach of winter. M'Gowan's theory of multiplication and reinfection is even less probable. According to this author, the chromatin granules in a spore escape by bursting of the spore, and play a part in transmission of the disease (scrapie) both endogenously and exogenously. Our knowledge of the Sarcosporidia indicates that the spore is the unit of

infection, and even if his theory were possible it would be hard to reconcile it with what is now known of the seasonal character of infection and reinfection. On the whole, considering in particular the work of Nègre, Erdmann and Crawley, it would appear that the source of infection is external and by the way of the alimentary canal. The experimental evidence brought out in this as well as in a preceding paper is in favor of the same conclusion. In fact, it is hard to see how seasonal infection could depend upon anything other than external conditions, either directly or indirectly, and since it is known that certain sarcosporidial spores enter the body by way of the alimentary canal, this is most likely the usual avenue of infection.

M'Gowan examined a large number of sheep and lambs, and incidentally produced some data that correlates with seasonal infection. Between January 21 and April 29, 1913, he found sarcocysts in 553 out of 818 sheep (67.6 per cent.), all of which were about one year of age or older. This shows that infection is the prevalent condition among sheep of that region. Between April 30 and June 11 he examined 121 February lambs and found sarcocysts in only four, or 3.3 per cent. Allowing forty to fifty days after infection for the sarcocysts to appear in the muscles, it is probable that this percentage would have been larger if there were no external conditions influencing the time of infection. At the same time the data agrees nicely with the idea of seasonal infection.

A discussion of some of the larger aspects of the relation of seasonal infection to the life history of *Sarcocystis tenella* will be deferred until a later paper. It seems entirely probable that infection occurs by way of the alimentary canal. It is clear that sheep at any age are susceptible to infection, and seasonal infection does not appear to be due to any condition within the sheep's body. Being so, do these conditions depend directly upon climatic factors, such as temperature? Or do they require an intermediate host present only at certain times of the year? The answer to these questions will depend much upon the answer to what is the life history of the parasite outside of the muscles of the host, a question which is yet unanswered. Successive seasonal infection is fatal to the theory of infection *in utero*. If there is a stage, or phase of *S. tenella* in the intestine which results in the freeing of spores, as Nègre (1907) has shown to be true of *S. muris*, it would seem that temperature may be a direct prominent factor in controlling seasonal infection. And yet since the encysted spores remained alive for thirty days in dried feces and resisted a considerable degree of heat, it does not seem possible on this theory to explain the entire absence of winter infection in *S. tenella* unless the spores are extremely sensitive to cold, which is improbable. There is no other

known climatic factor in the region of the Laramie Plains that could bear a direct causal relation to seasonal infection. Equally improbable on the same grounds is the theory that a carnivorous intermediate host is necessary. Other facts opposed to the latter view were presented in a former paper.

It would seem then that seasonal infection bears only an indirect relation to climate. If so, the factors that determine infection in turn must depend upon climate. To fulfil these conditions an intermediate or more likely, a definitive host is required. Crawley (1916) believes the Sarcosporidia should be classed under Telosporidia rather than as Neosporidia, basing his conclusion on what is now known of the life history of *S. muris* and certain young stages that he found of *S. tenella*. More needs to be known of the life history of these forms before this question can be settled definitely. Considering the widespread occurrence of Sarcosporidia in herbivorous animals, such as the sheep, Crawley also believes a second host is obligatory. This is probably correct, but his hypothesis with reference to a carnivorous animal must be rejected. Under the conditions of our experiment it is not possible to account for such infection as I have obtained on the theory of a carnivorous intermediate host, whether it be dog, cat, rat, mouse or ground-squirrel. If then a second host is necessary for *S. tenella*, the best remaining hypothesis is to look for this host among the invertebrates and a former paper has given reasons for believing this host would be found among the insects. The seasonal dependence of insects is also in accord with the facts presented in this paper.

There is a second hypothesis that must be taken into consideration. Nègre has shown that the feces of a mouse infected with *S. muris*, become and remain infective for a long time, from the fifteenth to the sixtieth day. Crawley has verified this work, and while the bodies that cause the infection have not been actually observed, they are known to exist in the feces. If there is a similar stage in the life-history of *S. tenella* the fragile character of the spores, which has been noted by Fantham and Porter, may be sufficient to account for seasonal infection. The dry, cool climate of this region, frequently becoming quite cold in the winter may soon kill the spores during a portion of the year. However, there are many mild periods in winter which should not be very destructive, and the lambs of the experiments noted in this paper ran, fed and watered with their ewes, so they had abundant opportunity to become infected by contamination. Yet there was not a single case of winter infection. Either the spores are set free at only certain seasons, or there is some doubt in regard to this second hypothesis. Experiments are now in progress which will probably show which of the two hypotheses is more nearly correct.

SUMMARY

The chief points of this paper may be summarized as follows:

1. There is a well-defined seasonal infection of *Sarcocystis tenella* in the region of the Laramie Plains. It is not known whether this is true or not of other regions. Young stages of this parasite have been found in the muscles of both sheep and lambs thruout summer and early autumn, but not during the winter and spring.

2. Reinfection occurs in successive seasons, and old sheep are apparently as susceptible to infection as are young lambs. The theory of infection *in utero* is untenable. Seasonal, self-reinfection is improbable, tho not entirely excluded, and the evidence indicates the origin *de novo* of successive infections.

3. If a second host is required, which seems probable, it is very likely that this host is an insect, and that the definitive (sexual) stage of the parasite will be found here.

4. If a second host is not necessary, the sexual stage probably takes place in the intestine of the sheep, and in some unknown way the life cycle falls under the influence of seasonal control.

5. In old ewes the larger sarcocysts are not nearly so abundant as the smaller ones. That some of the older sarcocysts do not grow to a large size is probably the most satisfactory explanation of this fact.

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THE EFFECT OF LAUNDERING UPON LICE (*PEDICULUS CORPORIS*) AND THEIR EGGS *

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At the request of Dr. Richard M. Pearce of the National Research Council, on July 25, the author took up the question of the effect of the ordinary steam laundry processes upon lice and their eggs. The object of the investigation was to determine to what extent these processes were destructive to both lice and eggs, and should they prove to be inefficient, what slight alterations could be made in the regular routine to make them effective.

LAUNDRY PROCESSES

Through the courtesy of Mr. J. Clair Stone, manager of the Elk Laundry, St. Paul, I was able to study the processes encountered in the washing of regulation army clothing. The clothing may be divided into three types: rough cotton goods (including cotton underwear), cotton khaki wear, and woolen goods (including garments part wool and part cotton). Altho the procedure differs somewhat in different steam laundries, they may in general be outlined as follows:

	Baths	Temperature	Time
Cotton Goods	1st Water	100° F. (37.7° C.)	5 min.
	2nd Neutral Soap	180° F. (82.2° C.)	15 min.
	3rd Neutral Soap	180° F. (82.2° C.)	15 min.
	4th Soda Bath	130° F. (54.4° C.)	10 min.
	5th Water	130° F. (54.4° C.)	5 min.

Cotton goods are dried in the hot air tumbler at a temperature of 150° F. (65.5° C.) to 190° F. (87.7° C.) until quite dry. Time about 20 minutes depending upon the load.

	Baths	Temperature	Time
Cotton Khaki	1st Water	100° F. (37.7° C.)	5 min.
	2nd Neutral Soap	120° F.-130° F. (48.8° C.-54.4° C.)	15-20 min.
	3rd Water	130° F. (54.4° C.)	5 min.

Dried in the hot air tumbler at 150° F. (65.5° C.) to 180° F. (82.2° C.) until just sufficient moisture is left in the garment that it may be pressed. Time about 10 to 15 minutes depending upon the size of the load. Pressed in the Universal Press.

	Baths	Temperature	Time
Woolen Goods	1st Neutral Soap	110° F.-115° F. (43.3° C.-46.1° C.)	15 min.
	2nd Water	110° F.-115° F. (43.3° C.-46.1° C.)	3 min.

Woolens are dried at room temperature and never in the hot air tumbler.

Work done at the suggestion and with the support of the Medical Division of the National Research Council.

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The first important point to determine was what effect the temperatures encountered would have upon the lice and nits. Data were available from the work of other investigations giving an indication of what results might be expected. The following table was taken from a compilation of Nuttall (1918).

IMMERSION OF EGGS IN HOT WATER

Temp., Degrees		Time	Result	Observer
F.	C.			
192	88	15 sec.	Killed	Nuttall
169	76	30 sec.	Killed	Nuttall
158	70	10 sec.	Killed	Nuttall
150	67	1 min.	Killed	Nuttall
140	60	5 min.	Killed	Nuttall
140	60	5 min.	Killed	Widmann
131	55	10 min.	Killed	Widmann
131	55	30 min.	Killed	Bacot
129	54	10 min.	Killed	Nuttall
121	50	15 min.	Killed	Widmann
112	45	15 min.	Not Killed	Widmann
104	40	1 day	Not Killed	Widmann

EXPOSURE OF EGGS TO DRY HEAT

124	51.5	15 min.	Not Killed	Experiments of Capt. Orr, Canadian A. M. C., and Bacot
127	53	15 min.	Not Killed	
130.5	55	30 min.	Killed	
132.5	56	20 min.	Killed	
134	57	30 min.	Killed	
152	57	15 min.	Killed	

EXPERIMENTS

In my experiments, it was found that the quantity of soap used varied somewhat due to the hardness of the water. Sufficient soap was added to the water to give a good suds. It was found that with the water used in the experiments recorded below that 1 gram of ivory soap (neutral) and $\frac{1}{3}$ gram of soda added to 265 c.c. of water furnished the desired suds. Inasmuch as the eggs are more difficult to destroy than the active stages, particular attention was paid to them. All the eggs were from lice collected from infected clothing, and kept in an incubator heated to 28° to 32° C. The eggs were laid upon small squares of cloth during the week of July 27 to August 2, in Exp. 1 to 6, and from July 27 to August 7 in Exp. 7 to 12. Each piece of cloth therefore represented eggs in different degrees of development.

Experiment 1.—Control set: 42 eggs; 78½% hatched.

Experiment 2.—Woolen Goods Treatment. Soaked in suds heated to 110° F.-114° F. (43.3 C.-45 C.) for 15 minutes. Rinsed in water of same temperature for 3 minutes, dried on a piece of filter paper and returned to the incubator; 65 eggs 92% hatched.

Experiment 3.—Khaki Wear Treatment. Soaked in suds heated to 121-126 F. (49-52.2 C.); average temperature, 123 F. (50.5 C.), for 15 minutes. Rinsed in water 123 F. (50.5 C.) for 4 minutes. Dried and returned to incubator; 38 eggs 39% hatched.

Experiment 4.—Khaki Wear Treatment. Same as Experiment 3 except treatment was for 30 minutes. 45 eggs, 0% hatched.

Experiment 5.—Cotton Goods Treatment.—Soaked in suds at 170-186 F. (76.6-85.5 C.), average temperature 179 F. (81.6 C.), for 30 minutes. Rinsed in water 130 F. (54.4 C.) for 5 minutes. Dried and returned to incubator; 52 eggs, 0% hatched.

The following experiments were conducted to determine the effect of treatment in the hot air tumbler and pressing in the Universal Press upon the eggs of the louse.

Experiment 6.—Eggs placed in pocket of a bathrobe in the hot air tumbler carrying a heavy load. Tumbler had been running for 5 minutes before eggs were placed in it. Eggs in the tumbler for 10 minutes and garments were quite moist when eggs were removed. Eggs replaced in incubator after treatment. 88 eggs, 0% hatched.

Experiment 7.—Control, 48 eggs. 100% hatched.

Experiment 8.—Cloth, upon which the eggs were laid, wet and then placed in the pocket of a pair of khaki trousers which was tumbled with others for 15 minutes. Load light and removed while still damp. Regular practice of drying khaki wear. 146 eggs, 0% hatched.

Experiment 9.—Eggs placed in pocket of partly dried bathrobe. Light load of clothing, tumbled for 10 minutes. 53 eggs, 0% hatched.

Experiment 10.—Same as Experiment 9, but tumbled for 15 minutes; 73 eggs, 0% hatched.

Experiment 11.—Same as Experiment 10, but tumbled for 20 minutes; clothing quite dry when removed. Regular cotton goods treatment. 57 eggs, 0% hatched.

Experiment 12.—Cloth with eggs placed under pocket of a pair of khaki trousers being pressed in the Universal Press. After treatment removed to incubator. 61 eggs, 0% hatched.

The recorded experiments upon the effect of soap suds at different temperatures upon the eggs of the lice would lead one to suppose that active stages would also be destroyed in those experiments where the suds had proved destructive to the eggs. To verify this, the following experiments were conducted.

Experiment 13.—Twelve recently fed lice in different stages of development were dipped in suds at 110-114 F. (43.3-45 C.) for 15 minutes, rinsed in water at 112 F. (44.4 C.) and dried on filter paper. All revived within a few hours.

Experiment 14.—Same as Experiment 13, but suds at 122-126 F. (50-52.2 C.), average temperature 124 F. (51.1 C.) for 15 minutes. All lice killed by treatment turning reddish brown within 5 hours.

Experiment 15.—Same as Experiment 14, but exposure lasting 30 minutes. All lice killed.

The experiments show that in the washing of rough cotton goods at 180° F.—82.2° C. for 15 or 30 minutes, will destroy the lice and their eggs. If by any chance an egg should escape destruction in the washing process they would later be destroyed during drying in the hot air tumbler. Washing cotton khaki clothing at a temperature of

120° to 130° F. (48.8° to 54.4° C.) for 15 minutes would prove destructive to the active stages, but would not completely destroy the eggs. Washing for 30 minutes, however, proved destructive to the eggs. Drying khaki uniforms in the hot air tumbler would also destroy any eggs that might have escaped the action of the hot suds. Pressing in the Universal Press was also effective, but this treatment cannot be relied upon to destroy all the eggs in an infested suit as portions of the uniform may not be touched. Neither the lice nor their eggs were destroyed in the woolen goods by the regular washing and since they are dried at room temperature, to avoid shrinkage, the problem resolved itself into devising some method of laundering woollens that would prove destructive. The first method which suggested itself was the treatment of the woolen goods in the hot air tumbler for 10 to 15 minutes before they are washed and while still dry. Nuttall (1918) claims "that the moderate degree of dry heat necessary to kill vermin will not prove injurious to wool, but that high temperatures 104° C. acting for 4 hours whilst but slightly yellowing white flannel does not affect its tensile strength, but if exposed to 127° C. for half an hour, flannel yellows and becomes brittle." This method, however, is open to two objections; namely, the danger of reinfestation of clean garments from handling garments infested with active stages in the vicinity of the tumbler, and the coagulating effect of the hot air on stains of blood, excreta, and other proteins, which may be present on garments before they are washed. Both these objections would be removed if the garments were first washed in such a manner as to destroy the active stages. The garments after drying could then be run in the tumbler to destroy all eggs which had escaped destruction during the washing.

In other experiments on contact insecticides (Moore and Graham, 1918) it had been found that where the insecticide possessed both wetting and spreading properties, the insecticide entered the tracheae of the insect, thus bringing about its death. Fat solvents, oils, etc., together with soap possessed such properties. Ivory soap, however, was found to possess great cohesion, thus preventing it from readily entering the tracheae. By raising the temperature of the solution or diluting it with water, the cohesion was reduced. From these results, it was not apparent why the suds used in the previous experiments (13) at a temperature of 110° F. to 114° F. (33.3° to 45° C.) should not have killed the active stages of the lice. The following experiments were conducted to throw more light on this point.

Experiment 16.—Lice not fed for 5 hours were dipped in a ivory soap solution of 1 gram to 100 c.c. of water colored blue with trypan blue. Temperature 108-115 F. (42.2-46.1 C.). Lice removed in 15 minutes and examined by mounting in alcohol on a glass slide, but no trace of the colored soap solution could be found in the tracheae.

Experiment 17.—Same as Experiment 16, but soap solution 1:250 c.c., results negative.

Experiment 18.—Same as Experiment 16, but soap solution 1:500 c.c., results negative.

Experiment 19.—Same as Experiment 16, but soap solution 1:750 c.c., results negative.

Experiment 20.—Same as Experiment 16, but soap solution 1:1,000 c.c., results negative.

Experiment 21.—Same as Experiment 18, but soap solution at a temperature of 122-132 F. (50-55.5 C.). Lice were killed by the treatment but no trace of the solution could be found in the tracheae.

Experiment 22.—Lice placed in soap solution 1:500 at room temperature at 8:13 a. m. and removed at 3:30 p. m. No trace of soap solution in tracheae of specimens examined. Lice divided into two lots; one rinsed in water; the other not rinsed. Both sets revived within an hour.

Since it appeared impossible for the ivory soap solution to enter the tracheae, a solution of castile soap with much lower cohesion was used but similar negative results were obtained. Soap solutions having failed to enter the tracheae, the question arose as to whether fat solvents or oils could penetrate.

Experiment 23.—Lice dipped in xylene stained with sudan III were examined at the end of 5 minutes but no trace of the stain could be found in the tracheae.

Experiment 24.—Lice dipped in ether stained with sudan III. One specimen examined after two minutes but no stain was detected. Stained ether was found in few tracheae of a louse in the ether for 5 minutes but none was found in a specimen removed after 8 minutes.

Experiment 25.—Twelve lice dipped in ether stained with sudan III for 10 minutes. Examination showed 7 with no ether in the tracheae and 5 which had ether in several tracheae but none with ether in all the tracheae.

Experiment 26.—Four lice dipped in a light lubricating oil stained with sudan III. Removed after 15 minutes, but no stain could be detected in the tracheae.

Most of the lice in these experiments were dead when removed from the liquid, having been killed by the chemical passing directly thru the body wall, since no stain could be detected in the alimentary canal. Landois (1865) has figured the closing apparatus of the pubic louse which is similar to that of the clothes louse and from the above experiments, the conclusion is reached that the louse is able to close this apparatus very quickly, but occasionally, as in the case of ether, a few tracheae are not closed quickly enough to keep out the chemical. A few experiments showed that the tracheae of the dog flea (*Pulex serraticeps*) was filled with stained ether after 1 minute immersion, but that the hog louse (*Haematopinus suis*) and the dog louse (*Haematopinus piliferus*) were somewhat resistant to its penetration, but not nearly so successful as the clothes louse. It is hoped to investigate this interesting observation more fully at some later date.

Two possible methods of killing the active stages is suggested by these results: First, the addition of a chemical to the washing suds capable of penetrating the chitin of the body wall during the period of washing, and toxic enough to produce its death, and second, the elevation of the temperature of the washing suds sufficiently high to destroy the lice. In general, a chemical capable of penetrating the body wall during the period of washing would have to be rather volatile and hence not suitable for the work. Judging from published accounts, soaking garments in a bath containing cresol or lysol is practiced to a large extent in Europe. The garments, however, are not rinsed following their dip. Peacock (1916) found a 1½ per cent. cold cresol solution to be capable of destroying the lice and nits soaked in it for one hour. Nuttall (1918) found a 5 per cent. cresol and soap solution to kill lice and nits in 30 minutes, while a 2 per cent. lysol solution at 76° F. (24.3° C.) killed the eggs after 5 minutes exposure. Bacot and Lloyd (1918) considers that "the evidence as a whole seems to establish the fact that steeping for twenty minutes in a 2 per cent. solution, either lysol or the cresol soap, is quite effective provided the temperature is not below 50° F." The following experiments were conducted to determine the efficacy of cresol either as a dip preceding washing, or when used in the wash suds.

Experiment 27.—Dipped 12 recently fed lice in suds with 1% tricresol added. Temperature 75 F. (24 C.). Removed after 5 minutes to suds at 110-114 F. (43.3-45 C.) for 15 minutes, rinsing in water at 112 F. (44.4 C.) for 3 minutes. Dried on filter paper when 10 lice revived.

Experiment 28.—Same as Experiment 27, but cresol suds at temperature of 110-114 F. (43.3-45 C.), 9 lice revived out of 16 used in the experiment.

Experiment 29.—Dipped recently fed lice in 1% tricresol in ivory soap suds at 110-114 F. (43.3-45 C.) for 15 minutes, rinsing in water at 112 F. (44.4 C.) for 3 minutes. Dried when one revived out of 17 lice.

Experiment 30.—Dipped in 2% tricresol in suds at 110-114 F. (43.3-45 C.) for 5 minutes. Placed in regular suds at 110-114 F. (43.3-45 C.) for 15 minutes, rinsing in water at 112 F. (44.4 C.). Dried, no lice revived.

Experiment 31.—Same as Experiment 30, but with 3% tricresol. All lice killed by the treatment.

From these results, it is apparent that 2 per cent. crude tricresol may be added to the washing suds or used as a dip preceding washing and prove effective in the destruction of the lice in the active stages. Although the pieces of cloth were rinsed after treatment, an odor of cresol persisted, apparently being rather difficult to remove.

Bacot and Lloyd (1918) point out that cresol emulsions are liable to decrease in insecticidal value in the presence of organic impurities. To what extent this action takes place is not known and possibly varies greatly. Such being the case and in view of the increased cost of

using a chemical to destroy the lice, further experiments were made to determine to what extent heat might be used. A summary of these experiments follows.

SUMMARY OF 30 MINUTE TREATMENTS

Temperatures	Dead	Revived
108-110 F., Average 108.8 F., 42.6 C.....	1	7
110-113 F., Average 110.7 F., 43.7 C.....	3	7
110-115 F., Average 111.6 F., 44.2 C.....	1	16
109-115 F., Average 112.4 F., 44.6 C.....	15	1
110-115 F., Average 113 F., 45 C.....	18	0
112-114 F., Average 113 F., 45 C.....	15	0

SUMMARY OF 22 MINUTE TREATMENTS

110-116 F., Average 112.8 F., 44.8 C.....	10	0
113-115 F., Average 114.2 F., 45.6 C.....	10	0
114-117 F., Average 115.2 F., 46.2 C.....	8	0

SUMMARY OF 15 MINUTE TREATMENTS

111-115 F., Average 112.3 F., 44.6 C.....	6	8
111-115 F., Average 113 F., 45 C.....	11	0
111-115 F., Average 113.3 F., 45 C.....	9	9
112-116 F., Average 114 F., 45.5 C.....	10	2
112.5-116 F., Average 114.2 F., 45.6 C....	18	0
113.5-117 F., Average 114.9 F., 46 C.....	8	0
115.5-117.5 F., Average 116.5 F., 46.9 C..	6	0

These experiments show the lethal temperature for lice is about 113° F. (45° C.) for 22 to 30 minute washings, and a slightly higher temperature 114.5° F. (45.8° C.) proved effective in 15 minutes' time. When woolen garments are quite soiled, the usual practice in laundries is to wash them at the higher temperature of 120° to 125° F. (48.8° to 51.6° C.), care being taken to keep the temperature constant thruout the process which is the important point in washing woolens to prevent shrinkage. These temperatures may be easily maintained in the washing machine.

Considering the data presented, the following procedure is recommended for the laundering of woolen goods to destroy both lice and eggs. Infested garments to be washed at a temperature of 120° F. (48.8° C.) not to fall below 115° F. (46.1° C.) during the washing period of 15 minutes, this treatment to destroy the active stages without the use of any special chemicals. Garments are then treated in the regular manner until perfectly dry, when they should be placed in the hot air tumbler at a temperature of 150° to 170° F. (65.5° C. to 76.6° C.) for 10 to 15 minutes resulting in the destruction of the eggs. By this method, it will be possible to launder woolens without shrinkage, and destroy the lice and eggs without the use of a special chemical.

These experiments have been corroborated in general by the experiments conducted with the regular army laundering units by W. Dwight Pierce and Lieut. A. Moscovitz. In their experiments, the woolens were washed at a slightly higher temperature, 131° F., and dried in the hot air tumbler without shrinkage resulting.

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THE ANATOMY OF *TETRACOTYLE ITURBEI* FAUST,
WITH A SYNOPSIS OF DESCRIBED TETRA-
COTYLIFORM LARVAE *

ERNEST CARROLL FAUST

Thru the courtesy of Professor Henry B. Ward, the writer has been enabled to examine specimens of *Planorbis guadelupensis* Sowerby infected with a new tetracotyle, to which the name *Tetracotyle iturbei* has been given (Faust 1918a). The material, for which I am greatly indebted to the kindness of Dr. Juan Iturbe, of Caracas, Venezuela, was at first considered to be "rediae" of *Schistosoma mansoni* (Iturbe and Gonzalez 1917). The infection occurs as cysts in the testicular cavities of the mullusk. As a result of the infection, these lumina are highly inflated, measuring two to three times normal size.

The material was examined by teasing out some of the worms and mounting as totos and by sectioning others *in situ*.

Description of *Tetracotyle iturbei* Faust 1918

Tetracotyle iturbei is a pear-shaped fluke measuring 0.42 mm. long, 0.33 mm. wide, and 0.3 mm. thick in the region of the primitive genital pore (Fig. 1). The oral sucker has a diameter of 52μ ; the primitive genital pore, 42μ , and the acetabulum 95μ . Posterior to the middle of the oral sucker and lateral in position are the accessory suckorial grooves with their oval openings directed anteromesad. These organs are undoubtedly muscular and are deeply sunken in the tissue of the worm. The body as a whole is enclosed in a thin mucoid cyst capsule, fitting tightly around the tetracotyle everywhere except in the region of the ventral attachment organs. There is no armament anywhere on the integument. No inclusive suckorial cup, such as is described for *Cercaria flabelliformis* (Faust 1918), is found to surround the ventral attachment organs. In sagittal section the outline of the worm resembles a similar section of a trochophore larva.

From the deeply sunken oral sucker the alimentary tract leads dorsad. Immediately above the oral sucker is the flask-shaped pharynx, 16μ in trans-section. The ceca arise at the dorsal end of the pharynx and proceed posteriad after looping somewhat ventrad and then dorsad again to the plane which the posterior end of the pharynx occupies.

* Contributions from the Zoological Laboratory of the University of Illinois, No. 125.

The ceca describe a wide furculum. They end blindly in the region of the anterior ventral sucker, or at times extend to the margin of the posterior ventral sucker. These diverticula are composed of a single layer of granular cells surrounding a small lumen. They have no suggestion of muscular elements. The cells of the ceca are cuboidal, about 4 to 5 μ thick, with spherical nuclei 2 μ in diameter, located in the center of the cells.

Only the main trunks of the excretory system were made out, and those with great difficulty. The material which was available for study was preserved in formol, so that none of the ultimate traces of the system were left. The bladder is situated dorsal to the genital pouch; it is nonmuscular and inconspicuous. Emptying into it are two swollen trunks which occupy the greater part of the body lateral to the digestive ceca. As far as they can be made out they have no cellular lining, but are merely lumina within the parenchymatous complex of the worm. These trunks extend as far anterior as the lateral suckorial grooves. They probably branch here, but the details cannot be followed. No excretory granules, such as are usually found in the Holostomidae, have been preserved in this specimen.

The nervous system of the holostomes has been remarkably altered, synchronously with the evolution of this group in other directions. The working out of this system was so difficult that Brandes (1891: 570) passed over its consideration in a brief paragraph, merely stating that he had observed nerve elements in the parenchyma of the suckers.

The present species has a nervous system similar in all essentials to that of *Cercaria ptychocheilus* (Faust 1918: 54, 55), but shows some interesting minor differences. In *Tetracotyle iturbei* the brain mass is large and is situated some distance dorsal to the pharynx (Fig. 4). There is a thick dorsal commissure. Anterior to it are dorsal and ventral trunks and perhaps traces of intermediate lateral trunks. From the posterior ventral angles of the brain are derived two paired trunks, dorsales and ventrales; and, in addition, a subesophageal commissure. The posterior dorsal trunk is fairly compact, cylindrical, and measures about 6 μ in cross section. On the other hand, the posterior ventral trunk is very diffuse, being much larger than the dorsal ramus, in all some 9 or 10 μ in cross section. The individual nerve fibers in this trunk can be easily distinguished. Given off from the ventral margin of the subesophageal commissure is a median fused trunk. In the plane of the lateral suckers it forks so that the branches of each fork surround the muscular region below the primitive genital pore. The muscular organ is the primitive vagina and the nerve is the genital nerve. In the region of the primitive genital pore the posterior dorsal trunks lie lateral and ventral to the ceca, while the large unsheathed ventrales lie in the plane of the pore, just lateral to it.

Branches of these two nerves form a dense mat between the parenchyma and muscle elements, ending in the body wall especially in the region of the acetabulum and lateral suckers. Prominent branches of the ventrales continue posteriad to the posterior genital pouch. The sensory nerve fibrillae (Fig. 3) pass in between the longitudinal and transverse nerve fibers and end in small papillae in the inner granular region of the integument.

The nerve cells are confined to the brain mass. No cell walls can be made out, but the nuclei are readily seen. They are oblong-ovate or reniform, and measure 1.5μ in short diameter and 4μ in long diameter.

The genital organs are differentiated early in holostomes. Midway between the acetabulum and the posterior genital pore is the ovarian cell mass, 25μ in transverse diameter. Dorsally, it opens thru the short oviduct into the ootype (Figs. 1, 5). The vitelline glands are long cylindrical cords, reaching cephalad as far as the primitive genital pore, and describing a broad H, with the anterior arms much the longer. At their anterior end the cords lie just ventral to the posterior limits of the ceca. Short transverse ducts connect them with the ootype. The vitellaria are composed of large granular cells with vesicular spherical nuclei. Posteriad, the ootype opens into a narrow cylindrical tube which continues caudad and opens to the exterior somewhat ventral to the excretory vesicle. There is no distinct enlargement into a genital pouch as has been described for various adult species and for the larvae, *Cercaria flabelliformis* and *C. pychocheilus* (Faust 1918: 110-112). Nor is there a definite muscular wall here. There are, however, numerous muscular elements which have their insertion posterior to the ootype and are spread out in fan-shaped arrangement, ending in the posterior body wall, muscles which undoubtedly serve in the capacity of dilating the genital atrium.

Pyriform testes, 50μ in trans-section, are found lateral to the ovary and slightly anterior to it. Their efferent ducts run caudad separately and open into the genital atrium from the sides. The testicular elements consist of polygonal cells with many chromioles and no well defined nuclei.

The anterior of the two ventral suckers is of the highest significance in the phylogenetic history of the holosomes. In the literature this attachment organ has been referred to as the acetabulum by Moulinié (1856) and later workers. The posterior ventral sucker has been regarded as an accessory suctorial organ, which most investigators have considered a creation *de novo*, but which Ssinitzin (1910: 19-21) has thought to be the modified genital pore. The latter investigator has seen a resemblance between the suctorial organ of *Holostomum erraticum* Duj. of Brandes (1891: Taf. 41, Fig. 5) and the

penial organ of his own unusual distome larva, *Cercaria plicata*. A most serious difficulty prevents such a conclusion, namely, that the genital organ of distomes is usually anterior or lateral to the acetabulum and in only a few species posterior to that organ.

Of all the writers on holostome anatomy and phylogeny, Odhner (1913) alone considers the posterior ventral sucker to be the acetabulum; the anterior ventral sucker he regards as a phylogenetically new organ. *Tetracotyle iturbei* provides ample evidence in support of the view previously proposed by the writer (1918) that the anterior ventral sucker is the primitive genital pore, while the posterior ventral sucker is the acetabulum.

A median sagittal section of the tetracotyle (Fig. 5) shows three openings on the ventral side, the oral and the two ventral suckers. The posterior ventral sucker is muscular. The outer part is funicular and leads into a large deep pocket which ends blindly ventral and caudal to the ovary. Likewise the anterior ventral sucker has a funicular opening. Within it there is a narrow tube, walled with a single thick layer of elongate cells leading dorsocaudad. Near the dorsal wall it opens into a U-shaped tube of large diameter lined with cuboidal cells of granular structure. This tube in turn opens into two genital organs, the ovary, caudoventrad, and the vagina, anteroventrad. The latter organ is large and irregular in contour, 37μ in thickness and 64μ long. It is walled with several muscular layers and has only a small lumen.

Hence *Tetracotyle iturbei* has two genital canals leading to the outside, one opening anterior to the ovary and just in front of the acetabulum, and the other opening caudad below the excretory pore. The genital canal opening thru the anterior ventral sucker proves this sucker to be a modified genital pore. On the basis of this direct evidence this sucker is to be regarded as the primitive genital pore of all tetracotyle and diplostomulum larvae, even where the connection with the genital organs has been lost. Furthermore, the undeveloped muscular elements of the posterior genital atrium in this species, together with the clear connection between the ootype and the primitive genital pore, suggest that this species is phylogenetically a transition form between distome and holostome types. The vagina is an organ not usually found in the holostome group. No Laurer's canal has been made out with certainty, but it probably arises from the dorsal wall of the glandular region along the primitive genital canal.

The study of the genital system in this species, then, contributes important evidence in support of the distome relationship of the holostomes. It shows the direct homology between the anterior ventral sucker of the holostome and the distome genital pore. In confirmation of Odhner's view it homologizes the posterior ventral sucker of holostomes with the distome acetabulum.

The encysted animal is covered with a thin but firm capsule of mucoid material of a bluish-gray hue. Beneath this is the integument. There is no epidermis present. Directly beneath the cyst capsule is a firm, almost homogeneous non-cellular layer, in which minute refractory granules are brought out by a very bright illumination. An equally thick layer of the same material lies just beneath this covering. It differs from the outer layer in being more diffuse and in having larger granules. The sensory nerve fibrillae penetrate into this layer and end in delicate papillae (Fig. 3).

In many regions of the body the parenchyma is almost obliterated by muscle and nerve elements. It may be stated with considerable certainty that little if any undifferentiated parenchyma remains in the larva at this stage of development. In the deeper regions of the body it has been converted into connective tissue. In the region next to the body wall the cells have long aciculate processes which penetrate thru the muscle layers into the inner integumentary layer. These cells probably function in the secretion of the integument.

The holostome larva as illustrated by *T. iturbei* is a unique example of muscular development. The muscles function primarily in the attachment of the worm to the tissues of the host and not in locomotion. The body wall has two series of muscles, an outer single layer of transverse fibers and many layers of longitudinal fibers just within the transverse layer. No muscles have been found in connection with the digestive ceca. The glandular elements of the acetabulum make it possible for this organ to function as a digestive organ. There is a strong pharyngeal sphincter around the esophagus, directly above the oral sucker.

The suctorial organs of this species all contain muscular elements. In most tetracotyles the acetabulum is described as possessing glandular elements. For the accessory lateral suckers of *Tetracotyle echinata* Diesing (1858: 367) and *T. petromyzontis* Brown (1899: 493, Fig. 5) definite granular structures have been described, but the glandular nature of these organs is probably of secondary origin and not their primary function.

Important retractor muscles are situated in two regions of the body. In the anterior part (Fig. 6), dorsal to the origin of the digestive ceca, a heavy double muscle band has its insertion. One part runs ventrad to the left of the pharynx and the other runs ventrad to the right of the pharynx. Each part of the band spreads out in fan-shaped arrangement so that it occupies the entire lateral region between the oral sucker and the genital pore. With the contraction of these muscles the entire region between these suckers is converted into a vacuum, by means of which the worm is intimately attached to the host. Muscle strips inserted in the region of the uterus of the worm have their

ending in the posterior wall. They probably function in the dilatation and contraction of the functional genital pore.

The muscle cell nuclei are usually spherical, with a diameter of about 4.5μ . In the region of the primitive genital pore, however, some are stellate. These nuclei are all abundantly filled with chromidia, which in some cases, are massed into karyosomes.

Only encysted individuals of *Tetracotyle iturbei* have been found. Like the distomes, the holostome larvae have been shown to be heterogenetic (Faust 1918). It is expected, therefore, that the cercariae of this species are produced parthenogenetically within a redia or sporocyst.

DISCUSSION

Tetracotyle iturbei is the first larval holostome to be described from South America. Records for North America have been made by Leidy, Rettger and Faust. These records, as well as those for *Tetracotyle typica* Europe are from molluscan hosts. Other tetracotyles are recorded from leeches, fish, amphibians, reptiles, birds and mammals. In every case except that of *Cercaria flabelliformis* the larvae have been found in the encysted or postencysted state. The doubtful case of *Tetracotyle hirudium* (Schomburgk 1844) gives the single record of an ectoparasite in the group.

No end of confusion in the systematology of holostome larvae has resulted from a disregard for the original diagnosis of the genera together with ignorance of the life-history processes of the group. The genera *Diplostomulum*, *Tyrodelpys*, and *Tetracotyle* have been recognized, but species of each of these have been placed in each of the other genera by overlooking items in the original description and by substituting incorrect descriptions for the genera to fit the cases in hand.

In 1832 von Nordmann proposed the name *Diplostomum* for the flat holostome larvae with two ventral suckers and no accessory lateral sucking organs. He recognized two subgenera with the type species *Diplostomum volvens* and *D. clavatum*. Unfortunately, he failed to name the subgenera. Diesing (1850: 304) removed the *clavatum* type to a new genus, for which he proposed the name *Tylodelphys*.

Tetracotyle typica was described by Steenstrup (1845: 129; Taf. 5, Fig. 3) as a "true distomata, *Distoma tarda*." The accessory suckorial organs were considered to be excretory organs. In 1855 de Filippi found the same species in conjunction with sporocysts of *Cercaria furcata*, and, recognizing the lateral organs as suckers, proposed the name *Tetracotyle* for the group. The name for the species described by Steenstrup and de Filippi, as proposed by Diesing, is *Tetracotyle typica* (1858: 366). In as far as this larval group can be recognized as a genus, this species may well be considered the type species.

The work of the writers on the present species, *Tetracotyle iturbei*, together with that on *Cercaria flabelliformis* and *Tetracotyle pipientis*, makes it possible to define the genus more carefully, without in the least deviating from de Filippi's original conception of the genus.

Redescription of Tetracotyle.—Holostome larva, oval, pyriform or ovate-oblong in contour, with ventral compression. Attachment apparatus consists of an oral sucker, ventral sucker (acetabulum) often degenerate and glandular, a ventral muscular genital pore usually somewhat larger than the acetabulum, and a pair of lateral suckers to the right and left of the pharynx, at times muscular, but also glandular in some species—all of these usually included within a muscular attachment cup. Primitive genital pore with or without functional connections with the genital organs. Excretory system having framework of an inconspicuous bladder, a pair of long cornuate vesicular trunks and a prominent transverse vessel which shifts its position in various species. Genital organs well differentiated in the larvae: consisting of a pair of oval testes, a pair of vitelline chorda, a median ovary and a posterior genital pouch. Nervous system highly modified. Parthenogenetic generations occurring in the mollusk, intermediate stage passed in vertebrates, and possibly in the case of *T. hirudinum* in leeches, and the definitive stage in higher vertebrates. Adult stage that in all cases to be the genus *Strigea*.

The genus *Tetracotyle* is differentiated from *Diplostomulum* and *Tyrodelpys* by the presence of lateral grooves, which are primitively muscular, but at times glandular. These grooves may be situated at the anterolateral margin of the worm or may be ventrally placed. *Monocerca heterobranchi* Wedl has chitinous grooves at the anterior margins. It may represent a transition from the *Tetracotyle* to the *Tylodelphys* type. Were the internal anatomy of all the species better known, a more fundamental basis for classification would be afforded.

Synopsis of Described Species of *Tetracotyle*

1. *Tetracotyle (Distoma) crystallina* (Rud.) 1819

Outline oval; length 0.4 to 0.6 mm.; width 0.25 to 0.45 mm.; oral sucker 130 μ in diameter; primitive genital pore 140 μ in diameter, median ventral; acetabulum glandular, auriculate; lateral suckers with small spines, opening forward. Excretory bladder rhomboidal, canals meandering, branching anterior to primitive genital pore.

Encysted in muscles of *Rana*, *Bufo*, and *Pelias* (*Viperus*). Europe.

2. *Tetracotyle (Heptastomum) hirudinum* (Schomburgk) 1844

Outline pyriform; length 0.62 mm.; remainder of description quoted directly from Diesing (1858:370): "Acetabula quattuor limbo ciliata, ventralia maximum subcentrale, alterum minus postpositum versus marginem posticum, transverse elliptica, et duo multo minore longe elliptica parallela, cum acetabulo marginale in triangulum disposita." Since Schomburgk figured his fluke up-side-down, his "versus marginem posticum" means toward the anterior end, and "aperturæ genitales discretæ antrosum sitæ" should read "aperturæ genitales discretæ posticum." Two oval or reniform testes are figured behind the ovary. The main excretory trunk is median, extending to the region just behind the testes at which place the transverse canal is

formed. The lateral canals are given off near the base of the main trunk; they give rise to many tubules and capillaries laterally disposed.

Recorded as parasitic externally, also in the genital organs of *Nephelis vulgaris* and *Clepsine complanata*. Europe.

3. *Tetracotyle percae-fluviatilis* Moulinié 1856

Outline oval; length 0.38 to 0.88 mm.; width 0.3 to 0.5 mm.; oral sucker 60 μ ; primitive genital pore 80 to 100 μ , in the posterior half of the body; acetabulum small, inconspicuous; lateral suckers 66 by 133 μ , lateral to pharynx. Crura long, meandering to posterior part of the body. Bladder small, lateral excretory trunks filiform, transverse vessel just behind primitive genital pore, secondary laterals from transverse vessel coursing forward.

Encysted in region of heart, *Perca fluviatilis*. Europe.

This species is credited to von Linstow in Lühe (1909: 170).

4. *Tetracotyle typica* Diesing 1858

Outline ovate to pyriform; length 10 mm.; width 0.62 mm.; oral sucker 59 μ in diameter; primitive genital pore 79 μ in diameter; acetabulum glandular, very large; lateral suckers auricular, subequal to oral sucker. Esophagus long, crura with many lateral ceca. Bladder hemispherical, pore subterminal, excretory stems meandering, branching in region of primitive genital pore; no transverse canal described.

Found in Lymnaea, Planorbis and Paludina in Europe; reported from *Lymnaea catascopium* and *Physa heterostrophia* by Leidy for North America.

5. *Tetracotyle echinata* Diesing 1858

Outline oval; length 0.62 mm.; lateral suctorial grooves glandular, sparingly covered with spines 3 to 4 μ long; grooves subequal to oral sucker. Network of excretory granules.

Encysted in oval capsules 0.5 to 0.6 mm. thick, in peritoneum of *Leuciscus idus* and *Acerina cernua*. Europe.

6. *Tetracotyle foetorii* von Linstow 1876.

Top-shaped, with transverse constriction anterior to primitive genital pore; length 1 mm.; width 0.48 mm.; oral sucker 130 μ in diameter; primitive genital pore 170 μ in diameter; acetabulum large, irregular, glandular; lateral suckers small, auricular. Crura from base of pharynx to region of acetabulum; large genital cell mass behind acetabulum.

Encysted in neck muscles of *Mustela (Foetorius) putorius*. Europe.

7. *Tetracotyle colubri* von Linstow 1877

Anterior end elongate, posterior end elongate—cylindrical; few large spines with broad bases on surface of integument; length 0.54 mm.; width 0.3 mm.; oral sucker 78 μ in diameter; primitive genital pore 120 μ in diameter; acetabulum considerably larger than primitive genital pore; lateral suckers oval, lateral to oral sucker. Crura arising from base of pharynx, extending to posterior end of primitive genital pore.

In thick-walled capsules embedded in subcuticula, *Tripidonotus (Coluber) natrix* and *Pelias (Vulperus) berus*. Europe.

8. *Tetracotyle soricis* von Linstow 1877

Similar in most respects to *T. colubri*. In capsules 1.2 mm. by 0.54 mm.; oral sucker 66 μ in diameter; primitive genital pore 110 μ .

Embedded in connective tissue in a double capsule, *Sorex vulgaris*. Europe.

This description is inadequate to warrant the creation of this species, but future work on the species may show it to be well founded.

9. *Tetracotyle ovata* von Linstow 1877

Outline large oval; spines confined to suckers; length 0.84 mm.; width 0.57 mm.; oral sucker 98 μ in diameter; primitive genital pore 130 μ in diameter; acetabulum 160 to 210 μ in diameter, opening backward ("larval anus" of von Linstow); lateral suckers elongate oval. Concentric rows of teeth on oral sucker and primitive genital pore.

Encysted in gut or peritoneum, or free capsules in body cavity, *Abramis (Blicca) bjoerkna*, *Osmerus eperlanus*, *Acerina cernua*, and *Abramis brama*. Europe.

10. *Tetracotyle lenticola* (von Linstow) 1878

Outline broadly pyriform; length 0.55 mm.; width 0.46 mm.; oral sucker 66 μ in diameter; primitive genital pore 66 μ in diameter; acetabulum about 60 μ in diameter, with many radiating glands; lateral suckers at extreme anterolateral reaches, consisting of lenticular muscular grooves. Excretory bladder triangular, vesicular; lateral canals constricted, with racemose tubules throughout body. Digestive crura to region just anterior to excretory bladder.

In lens, *Abramis vimba*. Europe.

11. *Tetracotyle petromyzontis* Brown 1899

This species was first found by Müller in 1840 and described as a diplostome in the fourth brain ventricle of *Petromyzon fluviatilis*.

Synonymy:—*Diplostomum* of *Petromyzon fluviatilis* Müller 1840

Diplostomum petromyzi fluviatilis Diesing 1850

Tylodelphys petromyzontis fluviatilis Diesing 1858

Diplostomum mülleri Cobbold 1860.

Tylodelphys petromyzi fluviatilis von Linstow 1878

Tetracotyle petromyzontis Brown 1899

Outline ovate, with oral end set off from body; length 0.42 mm.; oral sucker an ovoid cup; primitive genital pore slightly larger than oral sucker; acetabulum a longitudinal slit; lateral suckers auricular, multiglandular, just lateral to mouth. Pharynx powerful, ceca extending to subcaudal region. Genital cells consist of undifferentiated nuclear aggregates in region of primitive genital pore. Excretory bladder bicornuate, anterior tubules dendritic, posterior tubules prominently reflexed; transverse vessel split into two parts.

In fourth brain cavity of *Ammocetes*. Europe.

Leydig arranged his species *Tylodelphys crainaria* with Henle's *T. rhachiaea* and Müller's *Tetracotyle* of *Petromyzon fluviatilis* since they possessed in common "calcareous granules" within the body tissues of the worms.

12. *Tetracotyle phoxini* nov. spec

Synonymy:—*Tetracotyle* from *Phoxinus laevis* Mataré 1910.

Outline pyriform, with constriction separating anterior and posterior parts of body; length 0.2 mm.; width 0.15 mm.; oral sucker and primitive genital pore subequal; acetabulum larger, midway between primitive genital pore and bladder; lateral suckers auricular lappets, to right and left of oral sucker. Pharynx well developed, embracing entire esophagus; ceca extending to acetabular region. Excretory bladder large, bicornuate; split longitudinal canals, with a transverse canal in region of primitive genital pore.

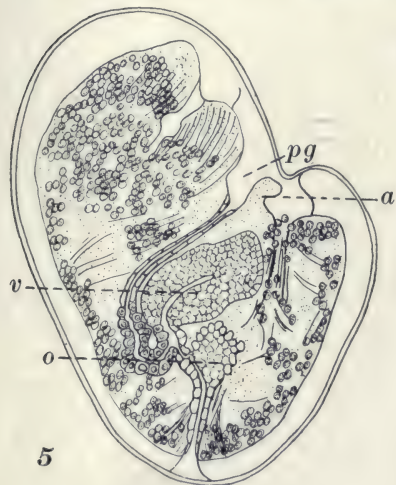
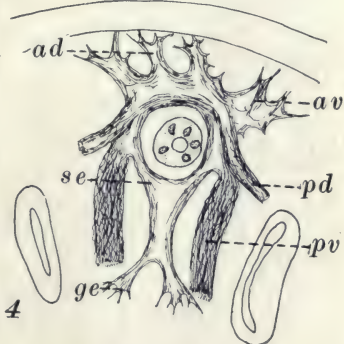
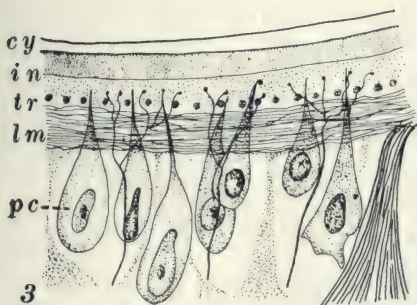
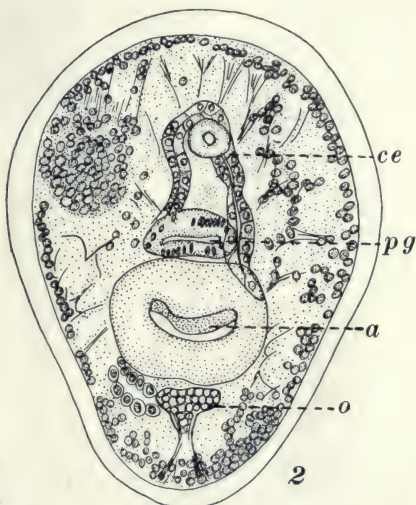
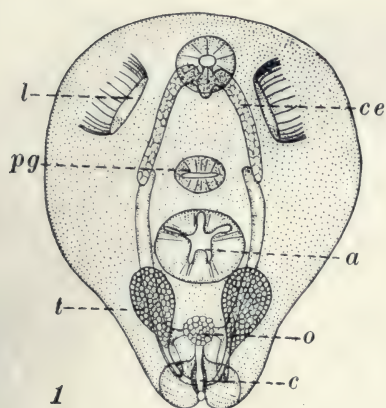
In brain and cranial cavity of *Phoxinus laevis*. Europe.

Mataré has brought together most of the true tetracotyles in his study, but he has also listed a number of diplostomula among these, as well as the agamodistoma of Wedl and of Leydig.

EXPLANATION OF PLATE

<i>a</i> , acetabulum	<i>o</i> , ovary
<i>ad</i> , anterior dorsalis nerve	<i>p</i> , pharynx
<i>av</i> , anterior ventralis nerve	<i>pc</i> , modified parenchyma cell
<i>c</i> , cirrus pouch	<i>pd</i> , posterior dorsalis nerve
<i>ce</i> , cecum	<i>pg</i> , primitive genital pore
<i>cy</i> , cyst capsule	<i>pv</i> , posterior ventralis nerve
<i>g</i> , posterior genital pore	<i>s</i> , subesophageal commissure
<i>ge</i> , genital nerve	<i>t</i> , testis
<i>in</i> , integument	<i>tr</i> , transverse muscles
<i>l</i> , lateral sucker	<i>v</i> , vagina
<i>lm</i> , longitudinal muscle	

Fig. 1. Ventral view of *Tetracotyle iturbei*, $\times 126$. 2. Frontal section of fluke thru digestive ceca, $\times 260$. 3. Region of integument and subintegumentary tissues, $\times 1500$. 4. Oblique frontal view of nervous system, $\times 396$. 5. Sagittal section of fluke, showing connection of vagina with primitive genital pore and posterior genital pore, $\times 260$. 6. Sagittal section of worm in region of pharynx, showing relation of retractor muscles to pharynx, $\times 396$.





13. *Cercaria (Tetracotyle) flabelliformis* Faust 1917

This is a tetracotyle in the pre-encysted stage. Its life history has been worked out thru the redia generation.

Outline ovate, with slight indication of caudal constriction; length 0.48 to 0.56 mm.; width 0.44 mm.; oral sucker 60 μ in diameter; primitive genital pore 50 μ in diameter; acetabulum confined to two transverse muscular lappets; lateral suckers oval in outline in young larva, wandering inward to sides of primitive genital pore and metamorphosing into lateral lappets in more mature larvae. Pharynx small; ceca sacculate, extending caudad two-thirds of body length. Excretory bladder inconspicuous; lateral canals with transverse vessel posterior to primitive genital pore; fan-shaped distribution of anterior tubules. Genital cell masses well defined, consisting of a club-shaped ovary, two vitellarian chorda, two testes posterior to ovary, and muscular genital cone.

In liver tissue, free or encysted, or in rediae, *Physa gyrina*. Corvallis, Montana.

14. *Tetracotyle pipientis* Faust 1918

Outline lyrate, with dense covering of spines; length 0.50 mm.; width 0.37 mm.; oral sucker 75 μ in diameter; primitive genital pore 80 μ in diameter, with a heavy crown of spines; acetabulum modified into a single transverse lappet; lateral suctorial organs elongate, obliquely placed, with large marginal spines. Pharynx small; ceca extending to center of primitive genital pore. Excretory bladder inconspicuous; lateral vessels, with transverse vessel far cephalad. Genital organs well defined, consisting of spherical ovary somewhat behind primitive genital pore, vitellaria in two diffuse chorda, two laterally disposed testes and ovoid genital cone.

Encysted in heavy capsules, mesentery and peritoneum, *Rana pipiens*, Chicago, Illinois.

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CONTRIBUCIÓN AL ESTUDIO DE LA PARASITOLOGIA EN VENEZUELA. ESTUDIO Y CLASIFICACIÓN DE UN DISTOMA

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El día 1º. de mayo de este año, haciendo la autopsia del cadaver de un enfermo procedente del Hospital Vargas, del servicio del Doctor M. A. Fonseca, mientras nos ocupábamos de buscar la ampolla de Vater para llevar a cabo la exploración del canal colédoco y del pancreático, siguiendo la técnica acostumbrada en la cátedra de Anatomía Patológica de esta ciudad, despues de habor abierto el duodeno entre dos pinzas y lavado con agua su mucosa, observamos en la superficie de esta membrana, al cabo de cierto tiempo de exploración con el estilete, un pequeño organismo que se movía, de aspecto al primer momento alargado, cilíndrico, de color oscuro, pero que en seguida pudo ser desplegado y mantenido extendido entre dos láminas de vidrio, apareciendo entonces con la forma de una hoja.

Resolví hacer el estudio y clasificación de este parásito, los cuales se encuentran expresados en las siguientes líneas.

DESCRIPCION

La observación del parásito me permite encontrar en él los siguientes caracteres:

Su cuerpo es de simetría bilateral. No está dividido en anillos, no es pues segmentado. No presenta miembros articulados. Es aplastado, delgado, desnudo, no recubierto de pestañas, foliáceo. En su contorno es más oscuro que el resto, excepto en un prolongamiento de él en forma de ángulo agudo en que no se nota ese tinte oscuro del contorno. Es ensanchado hacia el prolongamiento en forma de ángulo agudo de que he hablado y se estrecha para former este prolongamiento. El extremo opuesto a él que tiene la ventosa oral o bucal, de la cual hablaré más adelante, es redondeado o curvo. Es pues el conjunto del parásito semejant a una hoja: representando su prolongamiento angular, de que he hablado, el peciolo de esta hoja y el resto, el limbo; el cual resto tiene una forma de corazón.

Presenta dos ventosas: una ventosa oral o bucal y una ventosa ventral. La ventosa ventral está muy cerca de la ventosa oral. La ventosa bucal está en el vértice del prolongamiento del cuerpo en forma de ángulo agudo, del cual he hablado. La ventosa oral es más pequeña que la ventosa ventral. Las dos ventosas son redondeadas. La ventosa ventral tiene su abertura dirigida hacia la ventosa bucal.

Observo en seguida de la ventosa bucal un bulbo faringeano y un corto esófago. Este esófago es en forma de trapecio, sigue a dicho bulbo faringeano, menos teñido que dicha faringe, algo apagado; este trapecio tiene su pequeña base hacia el bulbo faringeano y su base mayor hacia la ventosa ventral; además este esófago es mucho más pequeño que el bulbo faringeano.

El tubo digestivo continúa el esófago, formando dos prolongamientos: uno que parte de un extremo de la base mayor del trapecio esofagiano y otro, del otro extremo de esta misma base. Estos prolongamientos se dirigen hacia el extremo del parásito opuesto a donde está situada la ventosa bucal; divergiendo (es decir alejándose el uno del otro) y guardando cada uno algún paralelismo con el borde que le queda más próximo de la extremidad del cuerpo del parásito en donde está la ventosa bucal; con dicha disposición continúan hasta llegar cada uno al lado y a alguna distancia de la ventosa ventral; entonces se inclinan algo hacia adentro; después a alguna distancia del sitio en que cambiaron de dirección, distancia que es próximamente igual a la que dista entre este sitio y su nacimiento en el esófago, estos dos prolongamientos divergen de nuevo hacia afuera, se alejan el uno del otro hasta terminarse cada uno en ramificaciones, que a su vez se ramifican. En el trayecto, desde que parten del trapecio esofagiano hasta que se resuelve en sus ramificaciones terminales, cada prolongamiento intestinal presenta ramas. En la porción de cada prolongamiento que guarda algún paralelismo con el borde que le queda más próximo de la extremidad del cuerpo del parásito donde está la ventosa bucal, hasta el sitio donde cambian de dirección al lado y a alguna distancia de la ventosa ventral, observo solo ramas que se dirigen hacia afuera.

Dimensiones tomadas después de hecha la preparación: Largo: Om., 0125. Mayor anchura: 0-, 007. Distancia entre la ventosa bucal y la ventosa ventral: Om., 0015. Pero parece haber sufrido algo de retracción.

Alrededor de la ventosa ventral, menos de la parte de este órgano que está hacia el esófago, se agrupan una gran cantidad de elementos de forma oval. Ya los otros caracteres que he observado y que he ya relatado me habían permitido hacer, por el estudio comparativo, la clasificación de este parásito; la cual se verá más adelante; y pude, por consiguiente, al observar estos elementos ovales, y mediante el mismo estudio, reconocer en ellos, por su forma y situación, los huevos del parásito.

CLASIFICACION

Por todos los caracteres que he observado en este parásito y su estudio comparativo me parece que puede decirse que pertenece a la ramificación de los Gusanos, clase de los Platelminotos, orden de los

Trematodes, sub-orden de los Distomianos, familia *Distomídeos*, género *Fasciola*, especie *hepatica*.

Fasciola hepatica tiene la siguiente "Sinonimia: Gran Distoma.—*Distomum hepaticum* Retzius 1786.—*Fasciola humana* Gmelin 1789.—*Distomum caviae* Sonsino 1890.—*Cladocoelium hepaticum* Stossich 1892."

"Este Gusano vive en los canales biliares del Carnero y del Buey; se lo ha igualmente observado, pero más raramente, en el Búfalo, la Cabra, el Camello, la Llama, el Caballo, et Asno, el Marrano, el Conejo doméstico (Railliet), el Conejo de conejar, la Liebre, el *Cobaye* (Sonsino) y en el Hombre" (Brumpt, 1913).

"Anatomía patológica.—Los Distomas irritan los canales biliares y producen una atrofia del tejido hepático; los canales biliares esclerosados hacen salida en la superficie del órgano y resaltan por su color blanco sobre el fondo rojo castaño del hígado...

Las lesiones histológicas son muy interesantes, el epitelio de los canales biliares prolifera y da nacimiento a adenomas biliares de un gran espesor. La pared es fuertemente esclerosada y la eosinofilia local en general muy marcada.

Distribución geográfica.—En Europa, *Fasciola hepatica* puede existir y aclimatarse por todas partes donde se encuentra *Limnaea truncatula* Müll. (*L. minuta* Drap.) que le sirve de huésped intermediario. Este Molusco es repartido en toda la Europa, el Thibet, el Asia Menor y el territorio del Amour (R. Blanchard). En la América del Sur, donde el Distoma es bastante repartido, su huésped intermediario es *Limnaea viator* de Orb.; en la América del Norte, este huésped es *L. humilis* Say; en las islas Sandwich: *L. oahuensis* Souleyet, y *L. rubella* Lea (Verdun). Es probable que el gran Distoma posee igualmente la posibilidad de evolucionar en otras especies de Limneas exóticas.

Papel patógeno.—El gran Distoma es un parásito raro y enteramente accidental en el Hombre, que ciertamente no le ofrece buenas condiciones para continuar su evolución. En los animales domésticos, este Gusano ocasiona una anemia perniciosa, conocida de los veterinarios bajo el nombre de *podredumbre* o de *caquexia acuosa*, de la cual el diagnóstico se hace fácilmente por la investigación de los huevos en las materias fecales (Brumpt, 1913).

Brumpt, a quien cito actualmente, habla también del diagnóstico por el precipito-diagnóstico y la fijación del complemento.

"El gran Distoma no ha estado observado que una veintena de veces en el Hombre: en el hígado, en la sangre, en el pulmón y en los abscesos sub-cutáneos. . . ."

Se dice que los jóvenes Distomas, fijándose en la cavidad bucal y en la faringe, producen una enfermedad llamada *Distomatosis buco-faringea*. Brumpt, refiriéndose a esta enfermedad, dice: "Dos jóvenes

Perros nutridos durante más de un mes de hígados de Carnero encerrando millares de jóvenes *Fasciola* y *Dicrocoelium* no me han jamás mostrado ningún parásito fijado sobre la mucosa bucal. Yo creo pues que sería necesario estudiar de nuevo la etiología de esta curiosa enfermedad, pues el papel de los Distomas me parece bien dudoso."

Verdun, refiriéndose a la *Fasciola hepatica*, dice: "En el Hombre, la distomatosis hepática, debida a esta especie, es más bien rara, pero se muestra siempre como una afección grave" (Verdun, 1913).

LA AUTOPSIA

Datos previos.—Cadaver N°. 918. Procedente del Hospital Vargas. Sericio del Doctor M. A. Fonseca. Sala N°. 9. Cama N°. 10. Diagnóstico clínico: Parasitosis intestinal. Hora del fallecimiento: las 9 a. m. del día 1°. del presente mes. Entró a la Escuela de Medicina el 1°. de mayo de 1918, a las 10 y media a. m. Nombre R. D.

Resumen del protocolo de la autopsia, el cual es el N°. 288.—Aspecto: enflaquecido. Sexo: masculino. Edad aproximada: 40 a 50 años. Tamaño: 1m., 65. Pericardio: con derrame. Corazón: normal, tiene 225 gramos de peso. Pulmones: el izquierdo adherente, antracósico, se nota en varias partes zonas de endurecimiento, congestionado, pesa 515 gramos; el derecho es adherente, con cavernas en la base y muy congestionado, pesa 530 gramos. Ascitis. El diafragma es descendido a la izquierda y a la derecha. Gran epiplón: retraído. Bazo: pequeño, cápsula arrugada, pesa 60 gramos. Riñón derecho pesa 140 gramos. Hígado: con adherencias en la cara superior, pesa 1300 gramos. Se encontró en el duodeno el Distoma que he estudiado y clasificado y el *Ankylostoma americanum* o *Necator americanus*.

Al preparar este trabajo, he leído las siguientes importantes publicaciones nacionales, referentes a Distoma y Distomatosis: Estado actual de la Parasitología en Venezuela por el Doctor Jesús Rafael Rísquez, Distoma y distomatosis en Venezuela por el mismo autor, Sobre Distomatosis Hepáticas en Venezuela por el Doctor Horacio Bello y Revisión de nuestras (?) Distomatosis hepáticas por el Doctor J. B. Ascanio Rodríguez. Pero no he tenido noticia de que en Venezuela se haya encontrado otra vez en la autopsia este Distoma a que me refiero, en el estado adulto, en el organismo humano.

Caracas, 19 de mayo de 1918.

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FLIES OF THE GENUS DROSOPHILA AS POSSIBLE DISEASE CARRIERS

A. H. STURTEVANT

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It was pointed out by Howard (1900) that the habits of certain species of *Drosophila* are such as to make them possible carriers of typhoid fever or other diseases. It is the purpose of the present paper to record certain observations bearing on this possibility.

Drosophila melanogaster Meigen (*ampelophila* Loew).^{*}—This cosmopolitan species was bred from human excrement by Howard, and there are a few other such breeding records from tropical regions; but I am very doubtful of the specific determinations in the latter cases. My own observations in the American tropics indicate that *D. melanogaster* is there extremely rare as an excrement fly, while *D. caribbea* (see below), which resembles it very closely, is common about excrement. Howard's breeding record almost certainly rests on a correct specific determination; and, this being the case, the habits of the adult flies are such as to make them open to suspicion, for *D. melanogaster* is always common about unprotected fruit in grocery stores and houses. Nevertheless, the species is a decided rarity about excrement, usually breeding in decaying fruit, and so is probably not an efficient disease carrier.

Drosophila caribbea Sturtevant.—This species, common throughout the American tropics, has habits very similar to those of *D. melanogaster*, both in larval and in adult life, but is much more frequently attracted to excrement. In Panama I have found it not uncommon about such material; and in Havana, Cuba, Mr. J. R. Taylor, of Las Animas Hospital, showed me specimens bred from the feces of a dysentery patient.

Drosophila busckii Coquillett, and *D. funebris* Fabricius.—These two species, both widely distributed and probably cosmopolitan, were both recorded by Howard as caught on human excrement. It seems probable from their habits that they would breed on such material; but they are not likely to be important as disease carriers, since they are not common about food. *D. busckii* frequently breeds on potatoes and other foodstuffs, but not until they are seriously decayed.

^{*}The writer has in press (Bull. Amer. Mus. Nat. Hist.) a synopsis of the Nearctic species of *Drosophila*, containing keys that include all the species of the genus known from the United States.

D. repleta Wollaston.—This species is common from Massachusetts and Indiana, south to Brazil, and also occurs in the Old World. Unlike many other species of the genus, it is most frequent near houses. It is attracted to various organic substances, and it has the peculiar habit of coming to rest frequently on a white surface. In the eastern states the form is most easily collected about urinals that are not kept clean or thoroughly disinfected—such places as are frequently to be found in saloons or railway stations. The next most likely place to find the species is in kitchens or restaurants, especially on bread or on white walls or tablecloths. I have seen it frequently both in restaurants and in urinals in Boston, New York, Washington and elsewhere.

In Cuba *D. repleta* literally swarms around any place where excrement is allowed to remain in quantity. It is by far the commonest fly in such places, as Dr. C. W. Metz and I have observed at Guines, Aguada Pasajeros and elsewhere. Isolated deposits are not favorable, being usually attacked chiefly by species of Leptocera (*Limosina*) and Sepsis—which forms are practically never found about food and are therefore not dangerous.

D. repleta has a wide range of breeding habits, so that control measures would be difficult. It breeds on various kinds of fruit (banana, pineapple, tomato, etc.), though it is not so common on fruit as are several other species of the genus. It will also breed on decayed potatoes, flour paste, moist bran, and various similar substances. Although it has not been bred from excrement, there can be little doubt that it does use such material for larval food.

The literature would lead one to suspect *Drosophila melanogaster* as the most dangerous species, with *D. funebris* and *D. busckii* of doubtful significance; but more detailed observations lead to the conclusion that none of these three species can be particularly dangerous, whereas *D. repleta*, and *D. caribbea* in the tropics have habits of such a sort as to make them important as possible disease carriers.

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A NEW CERCARIAEUM FROM NORTH AMERICA *

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University of California

During July and August, 1915, while studying at the University of Michigan Biological Station at Douglas Lake, Michigan, rediae containing tailless cercariae were found in the livers of nine out of twenty-six specimens examined of *Planorbis campanulatus smithii* Baker. Since the adult of this species of cercaria is not known, I will place it in the provisional generic group *Cercariaeum* and give it the name *Cercariaeum mutabile*.

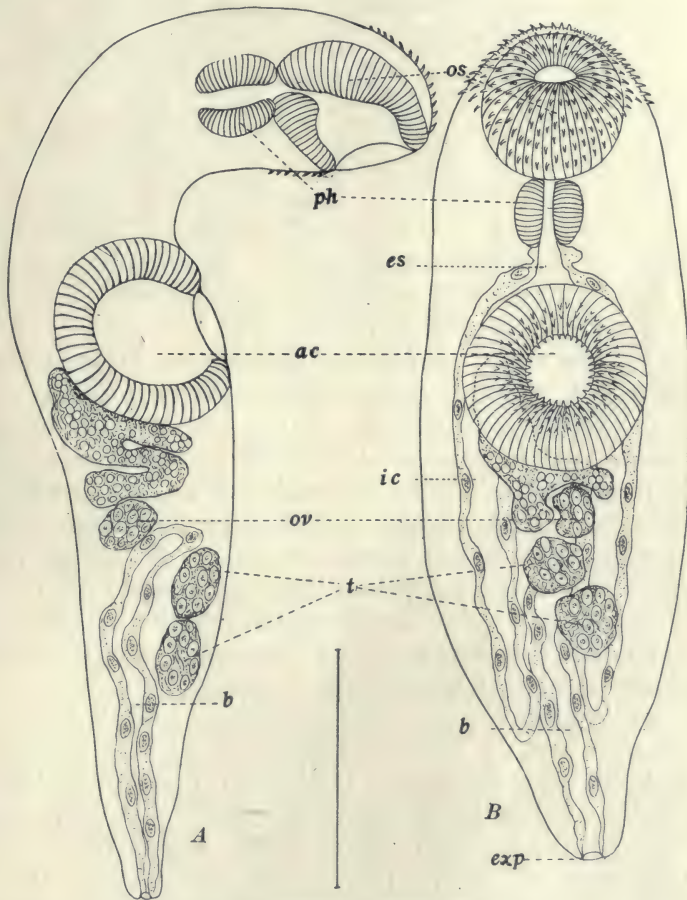
The hosts were collected from shallow water along the shore of the lake. The rediae (Figs. 1 and 2) filled the liver of the infected host and contained cercariae in all stages of development. The smaller rediae were quite mobile, altho they had no locomotor appendages. Since the rediae were without birth pores, the cercariae were obtained for study by breaking them open. The digestive sac of the redia (*ds*) is short, reaching even in the younger specimens to less than a third of the total length. The excretory system of the redia is divided into two entirely distinct halves. From the excretory pores (*exp*) short bladder tubes (*bt*) run forward which bifurcate into collecting tubes (*ct*) extending forward and backward. The anterior collecting tubes receive capillaries (*c*) from three flame cells (*f*) on each side, while the posterior collecting tubes receive the capillaries from two flame cells. The excretory system of this redia was very difficult to work out and I am not sure that all the flame cells present were located.

Cercariaeum (mutabile) (Text-figure A and B) is a large form with the adult characters well developed and almost no adaptive larval characters. No trace of a tail could be found at any stage of development of the cercaria. This cercaria is very mobile, being able to extend and contract its body to a remarkable extent. At greatest extension the body becomes so long and narrow that it resembles a nematode except for the large acetabulum which juts out prominently. When at greatest contraction the body is almost round and the acetabulum is pulled up against the oral sucker. The cercaria moves actively on a substratum by the use of its suckers, but is unable to swim.

The suckers of *Cercariaeum mutabile* are large and powerful. The acetabulum is the larger, having a ratio to the oral sucker of about three to two. The cuticular spines (Text figure B) cover a very limited area of the anterior tip. They are also found in several rows

* Publication from the University of Michigan Biological Station.

surrounding the opening of the acetabulum. The digestive system consists of a large muscular pharynx (*ph*), a short esophagus (*es*) and intestinal ceca (*c*), which reach almost to the posterior end of the body.



Cercariaeum mutabile

A, side view; B, ventral view; *os*, oral sucker; *ph*, pharynx; *ac*, acetabulum; *ov*, ovary; *t*, testes; *b*, excretory bladder; *es*, esophagus; *ic*, intestinal cecum; *exp*, excretory pore. Scale equals 0.1 mm.

The excretory system of *Cercariaeum mutabile* (Fig. 3) consists of a single club-shaped bladder, a complicated series of collecting tubes and sixty-four flame cells with their capillaries, arranged in eight groups of four on each side. On the left side (Fig. 3) the flame cells and their capillaries are not shown. The figure is drawn from the dorsal side and the accessory collecting tubes, the capillaries and flame cells which supply the ventral side are shown in dotted lines. The principal collecting tubes on each side divide each into two tubes, the

posterior (a') of which is much longer than the anterior. The subdivisions of these two tubes (a and a') correspond except that the relations are reversed. It can be seen that of the subdivisions of a which I have designated b and c , the one which extends posteriorly (b) does not further subdivide, while of the subdivisions of a' which are designated b' and c' it is the one which extends toward the anterior end (b') which does not again subdivide. This same relation is carried out in the third subdivision (cf , d and d' and e and e'). The capillaries from the flame cells join the accessory collecting tubes in definite groups of four, half of which are dorsal and half ventral. The flame cells are so distributed that every region of the body is drained. The extent of the subdivisions of the collecting tubes, the large number of flame cells and the definite arrangement of the capillaries into groups suggest that the excretory system of this cercaria is fully developed and represents the adult condition for the species.

The excretory system of *Allocreadium isoporum* (Looss) described by Looss (1894: 51-52, pl. 5, fig. 103) resembles in certain striking particulars the system just described for *Cercariaeum mutabile*. In *Allocreadium isoporum* the number of flame cells in each capillary group is four and the character of the bladder and the position of the main collecting tubes is the same as in my species. There are differences in the total number of capillary groups of which there are only six on each side in *Allocreadium isoporum*, and also in the arrangement of the accessory collecting tubes. The fundamental resemblances between the excretory systems of these two species must in my opinion indicate some degree of relationship.

The reproductive system of *Cercariaeum mutabile* is so far along in development that the adult arrangement of the organs can be partially made out (Text figure A). The testes (t) are located diagonally one behind the other along the longitudinal axis of the body about the middle of the post-acetabular region. They are ventral in position while the ovary (ov) which is just in front of them is near the dorsal surface. I was unable to clearly define the outlines of the other reproductive organs or to be certain of the location of the genital pore.

DISCUSSION

Since the provisional genus *Cercariaeum* is based on the single character of the absence of a tail in the fully developed cercaria within the sporocyst or redia, it is evidently not a natural group. The loss of the tail would seem to be due to the degeneration of this organ following the adoption of a type of life history in which the free swimming state is omitted. Such an adaptation might arise in any group of digenetic trematodes. Species which may be correctly placed in this provisional genus should be carefully distinguished from free

agamodistomes, which are larval distomes which have escaped from their sporocysts or redia and are waiting unencysted in secondary intermediate hosts to be carried into their final hosts. Sometimes a cercaria becomes an agamodistome in the same host which harbors its sporocyst or redia. This is the case with the so-called *Cercariaeum helicis* (Leidy), found in species of genus *Helix*. Hofmann (1899) finds that the cercaria of this species develops in sporocysts in the tissues of the snail host. This cercaria, which has a very degenerate tail, escapes from the sporocyst and migrates into the kidney of the same snail where it lives as a free agamodistome until it is carried passively into its final host. Such a life history shows the free life of the cercaria reduced to a passage from one organ of the snail to another. This life history approaches the condition found in the *Cercariaeum* group in which the free stage is very probably entirely omitted from the life history.

Two species of the provisional genus *Cercariaeum*, *Cercariaeum limnaei obscuri* Ercolani and *Cercariaeum paludinae impurae* Filippi (see Lühe, 1909, 208) resemble *Cercariaeum mutabile*. Lühe (1909, 93) refers the second of these cercariae to the species *Asymphylodera tincae* and suggests that the other belongs to some *Asymphylodera* species. The structure of these forms is not fully enough described to make a detailed comparison possible. *Cercariaeum mutabile* differs from *Cercariaeum paludinae impurae* in spination, in the size of the digestive sac of the redia and in the length of the esophagus of the cercaria. Further its structure is very different from that of the members of the genus *Asymphylodera* which have only one testis and a very small round excretory bladder.

Cercariaeum mutabile in contrast with such types of larval trematodes as the schistosome or stylet cercariae shows a considerable development of adult structures and practically no adaptive larval characters. The contrast is very striking between this cercaria and such a form as the cercaria of *S. japonicum* (Cort, 1918) in which adaptations for penetration dominate the whole structure, and adult characters are practically undeveloped. Since *Cercariaeum mutabile* has no adaptations for swimming, encystment or penetration, it seems very probable that there is no free swimming period in its development, and that it must be carried passively into some final host which feeds upon the snail intermediate host.

Altho I have no direct evidence in regard to the further development of my *Cercariaeum*, structural comparisons give some clue to its relationship. As stated above the similarity of the excretory system of *Cercariaeum mutabile* to that of *Allocreadium isoporum* (Looss)

EXPLANATION OF PLATE

Cercariaeum mutabile. Scale equals 0.1 mm.

Fig. 1. Redia showing contained cercariae; *ph*, pharynx; *ds*, digestive sac; *c*, fully developed cercaria; *uc*, undeveloped cercaria.

Fig. 2. Redia showing the excretory system; *exp*, excretory pore; *bt*, bladder tube; *ct*, collecting tube; *c*, capillary; *f*, flame cell.

Fig. 3. Excretory system, dorsal view. On the right side of the figure all parts of the excretory system are shown but on the left side the capillaries and flame cells are omitted. Anterior subdivisions of the collecting tube on the left side of the figure are labeled *a-f* and the corresponding posterior subdivisions *a'-f'*. Accessory collecting tubes, capillaries and flame cells of the ventral side are shown with dotted lines. Letters as in text figure; also, *mct*, main collecting tube; *act*, accessory collecting tube.

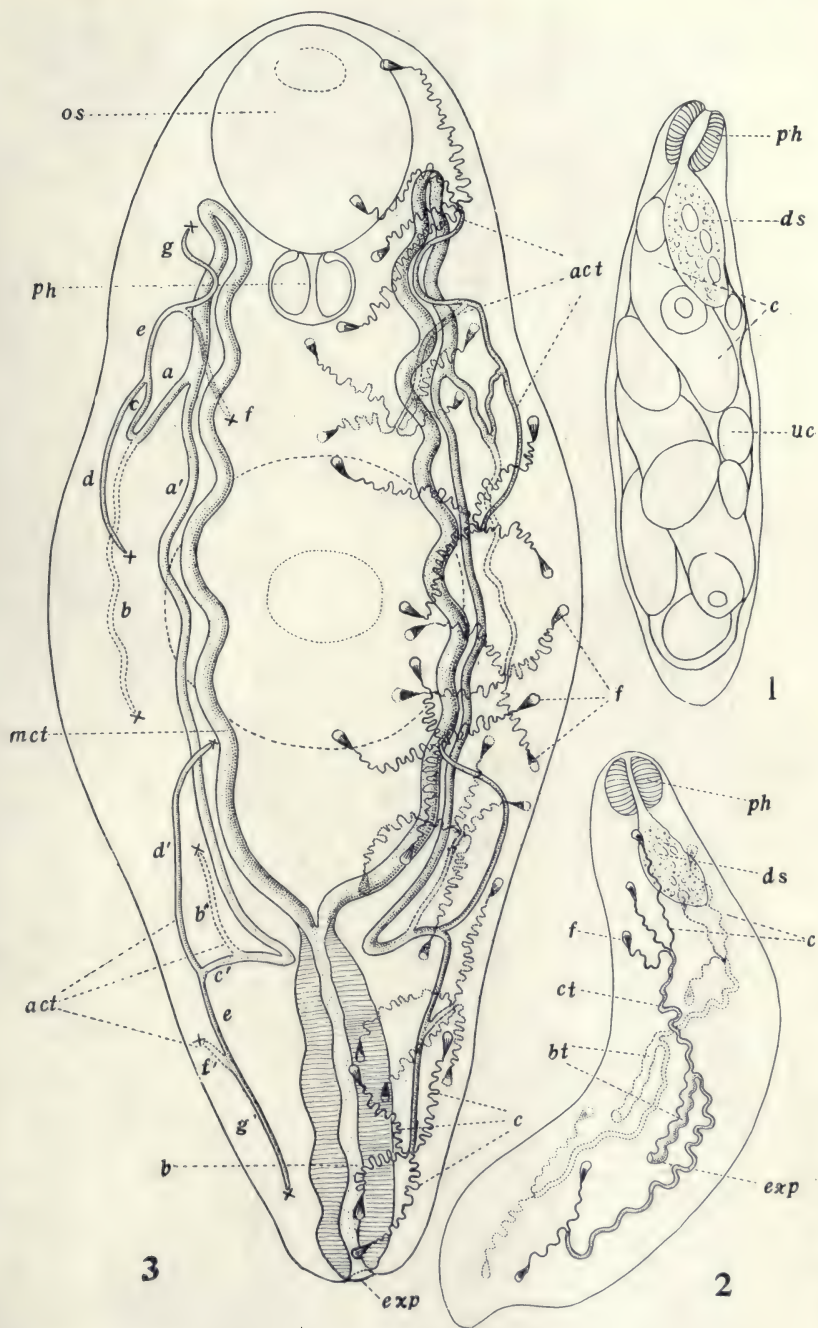
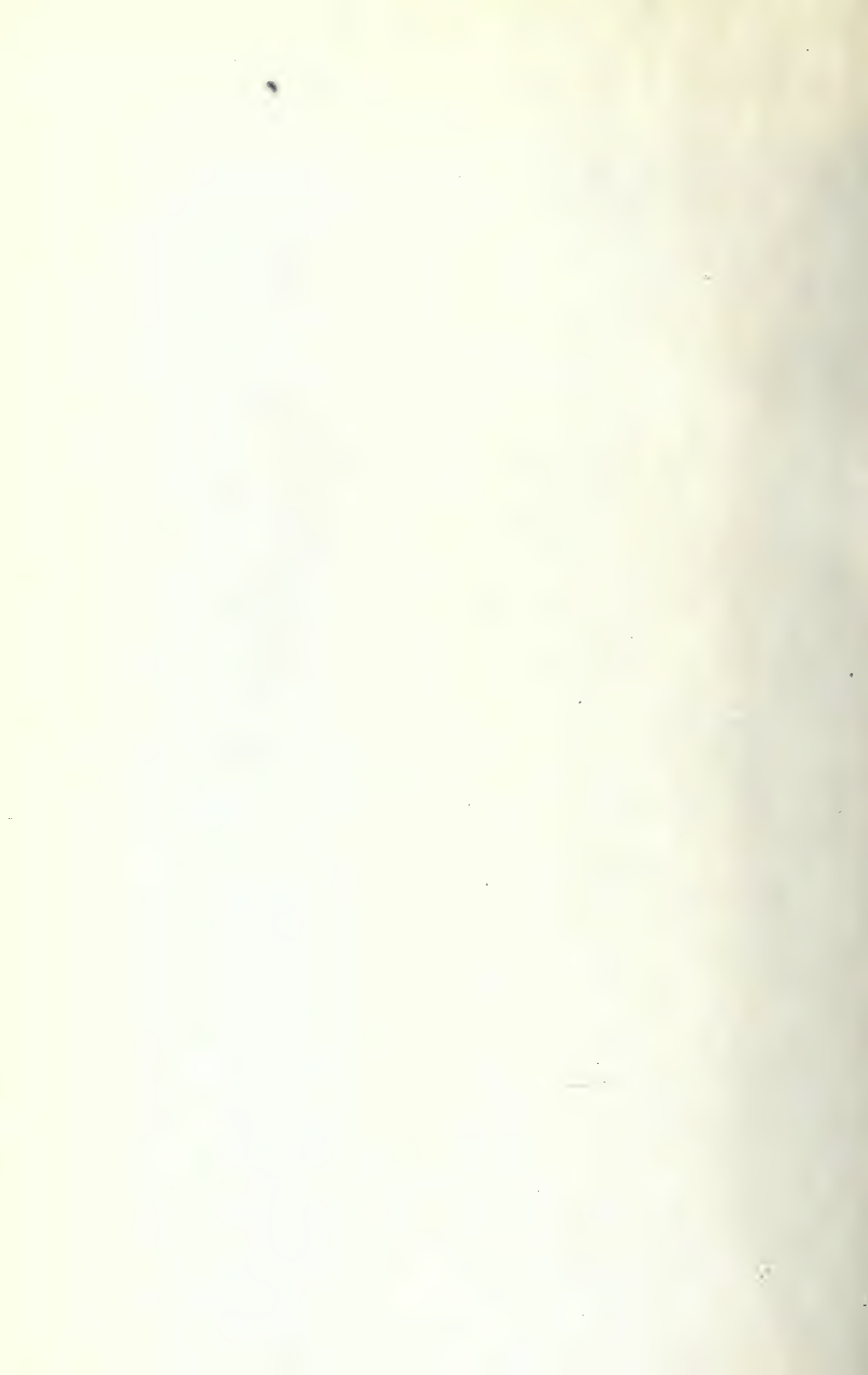


PLATE VII



seems to indicate relationship. Further, the large size of the ventral sucker of my species and the characteristics of its digestive and reproductive systems place it in the subfamily Allocreadiinae Odhner, or at least very close to this group.

SUMMARY

Cercariaeum mutabile is a new species described from *Planorbis campanulatus smithii* from Douglas Lake, Michigan.

This cercaria has practically no adaptive larval characters and a considerable development of adult characters, evidently correlated with the omission of the free swimming stage from its life history.

The excretory system consisting of a simple club-shaped bladder, a series of collecting tubes, and sixty-four flame cells with their capillaries arranged in eight groups of four on each side.

The adult of *Cercariaeum mutabile* is not known, but its structure relates it to the subfamily Allocreadiinae Odhner.

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REVIEWS AND NOTES

The recent receipt of the initial number, July, 1918, in the sixth volume of the Indian Journal of Medical Research leads naturally to a review of this periodical which has done notable work in the field of parasitology. Founded in July, 1913, as the official organ of the Indian Research Fund and devoted entirely to the publication of research work directly or indirectly connected with medical and sanitary science, it has published each year since four numbers of goodly size that are marked no less by the high character of the papers contained than by the attractive appearance and admirable illustrations they present.

From the start marked emphasis has been laid on parasitology by the amount of the material published in that field and in the first number half of the papers or more belong in that category. It would be impracticable here even to mention all the articles on various phases of medical zoology which have been printed in the first five volumes. But their varied character may be rightly estimated from the fact that the last (fifth) volume includes papers on the life cycle of *Schistosoma spindalis*, on atypical malarial parasites, Negri bodies, Kala-azar, entamebic cysts, *Ochromyia jejuna*, parasitic Muscidae, ancylostomes, spirochaetes, Trichomastix, mosquitoes, and others on insects as well as on topics less immediately related to the subject but no less interesting to the parasitologist. The regular and normal appearance of so extensive and valuable a series during the height of a world war demonstrates indubitably the permanence of the foundations on which it rests.

The Indian Research Fund Association is to be congratulated on having established and maintained a publication of such high rank among the medical scientific journals of the world. To investigators in parasitology it has become indispensable, and one cannot doubt that it has furnished both at home and abroad a real stimulus to the development of the subject that will show itself in an ever widening circle of workers and in a constantly growing series of contributions of importance.

Professor Raphael Blanchard, editor of the Archives de Parasitologie, which was printed at Lillie and has been suspended since 1914, desires an announcement made of the fact that when Lillie recently passed into the control of the French, part 4 of volume 16 of the Archives, completely printed and dated August 1, 1914, and the seven first signatures of volume 17, plates, cuts, manuscripts, etc., were found uninjured.

Part 4 of volume 16 will be distributed immediately and the Archives will again make its appearance regularly as soon as it is possible to establish conditions for its appearance.

Professor Blanchard's new address is 4, Avenue du Président Wilson, Paris, 8e.

Sanidad y Beneficencia, Boletín Oficial, of Havana, Cuba, has published as a double number a splendid memorial to Dr. Carlos J. Finlay. The number contains as frontispiece a portrait of this distinguished student of medicine and parasitology and includes some twenty articles on his work and the recognition it has received in various ways.

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RECENT DISCOVERIES CONCERNING THE LIFE HISTORY OF *ASCARIS LUMBRICOIDES* *

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Ascaris lumbricoides is one of the most common and most important intestinal parasites of man. A roundworm sometimes known as *Ascaris suum*, or *A. suilla*, but morphologically indistinguishable from *A. lumbricoides* and probably of the same species, is of very frequent occurrence in the intestine of the pig. Until recently it had been generally assumed by parasitologists upon the basis of evidence collected by various investigators that the life cycle of *Ascaris lumbricoides* is simple and direct, that the eggs of the parasite which pass out of the intestine of the host animal in the feces, are swallowed by another human being or pig after a period of incubation sufficient for the development of the contained embryos to a vermiform stage, and that having been swallowed the eggs hatch in the alimentary tract, after which the embryos develop to maturity in the small intestine, the normal location of the adult worms. Stewart, however, in a series of notable papers (1916-1918) has lately presented the results of some investigations which have revealed imperfections in our former ideas of the life history of *Ascaris lumbricoides*. His contributions to our knowledge of this common parasite afford another striking illustration of the fact that prevailing and apparently well established views as to the life histories of parasites are often wrong.

Stewart first attempted to infect pigs by feeding them *Ascaris* eggs but failed. He then fed the eggs to rats and mice, and discovered that they hatched out in the alimentary tract, a fact already established by Davaine (1863), who also noted that newly hatched larvae could be found in the feces of rats soon after feeding the eggs. Stewart observed further, however, that not all of the young worms are thus eliminated in the feces. On the contrary many of them penetrate the

* Read at the meeting of the American Society of Zoologists, December 26, 1918.

† W. D. Foster died October 6, 1918.

intestinal wall and, aided by the circulation, migrate to the liver, spleen and lungs, and may be found in the liver and lungs two to four days after infection. He determined that the migrating worms undergo considerable growth and development, and in fact increase in length from about 0.22 mm. to as much as 1.4 mm. within a week after infection. They can be found in the bronchi and trachea seven or eight days after infection, later in the mouth, then in the esophagus, stomach and intestine. Having reached the intestine after their migrations through the lungs the larvae linger for a time in the cecum, but ultimately pass out of the body in the feces without development beyond the stage already reached in the lungs, except that they may become slightly larger reaching a maximum length of nearly 2.5 mm. According to Stewart, a rat or mouse may become quite free of the parasites as early as 16 days after infection. During the invasion of the lungs by the worms Stewart found that rats and mice commonly died from pneumonia. Influenced by his failure to infect pigs through feeding them *Ascaris* eggs and by his discovery of the behavior of the larval parasites in rats and mice, Stewart concluded that these animals act as intermediate hosts, the young worms being passed on to human beings and pigs through the contamination of food, water, etc., by the saliva or feces of rats or mice that had themselves become infected by swallowing the eggs of the parasite. It is necessary to admit that infection of man or pig in this way is theoretically possible, but it appeared to the writers following the publication of Stewart's earlier papers, that this explanation of the mode of infection was inadequate.

We had for a number of years been carrying on certain investigations relating to *Ascaris* in which infested pigs were utilized, and had repeatedly attempted to secure heavily infested subjects by feeding the animals with *Ascaris* eggs, but with very unsatisfactory results, so that our early experience with pigs was very similar to Stewart's. Nevertheless the results of Stewart's experiments with rats and mice and his failures and our failures to infect pigs did not seem necessarily to lead to the conclusion that rats and mice normally serve as intermediate hosts of *Ascaris lumbricoides*. The questions raised by Stewart's investigations were highly important from a practical as well as a purely scientific standpoint, and it appeared desirable that the Bureau of Animal Industry should collect further data that might assist in reaching definite conclusions. Accordingly the present writers repeated and supplemented Stewart's experiments. Without going into all the details of our work at this time, it may be stated that we have confirmed Stewart's results as to the behavior of *Ascaris* larvae in rats and mice. We have, however, in addition, made obser-

vations that appear to us to demonstrate very clearly that rats and mice are not normal intermediate hosts as Stewart suggested. In our opinion the real explanation of the behavior of *Ascaris* larvae in rats and mice is that the worms are merely going through the same course as they do in their usual hosts, man and pig. The only essential difference in the two instances is that in unsuitable hosts such as rats and mice the parasites are unable to complete their development to maturity, whereas in human beings and pigs after their migration through the lungs and return to the alimentary tract they can continue their growth to the adult stage. The value of Stewart's investigations, therefore, lies in the establishment of certain important facts relating to the migration of *Ascaris* larvae and not in the suggestions that he has made with reference to rats and mice as intermediate hosts.

In guinea-pigs and rabbits we have found that the larvae behave as they do in rats and mice with respect to their development, migration and elimination, and the fact that they are liable to cause a more or less serious pneumonia. From a young goat and a lamb after feeding them eggs of the pig *Ascaris* we have recovered immature worms that had developed beyond any stage yet obtained from rats, mice, guinea-pigs, or rabbits. In the case of the lamb, which two days after birth was fed *Ascaris* eggs and killed 103 days after feeding, we found in the intestine fifty partially grown ascarids, twelve males and thirty-eight females, the smallest male 60 mm., the largest female 130 mm., in length. The minimum lengths of the adults are about 150 mm. (male) and 200 mm. (female). The goat four days after birth was given a dose of *Ascaris* eggs, and 17 days later, a second dose. Seven days after the second dose the animal began to show symptoms of pneumonia and died three days later. In the lungs, trachea, esophagus and stomach numerous *Ascaris* larvae were found ranging from 1 to 2 mm. in length. These undoubtedly are traceable to the second feeding with *Ascaris* eggs, 10 days previously. In the small intestine were thousands of young ascarids measuring about 10 mm. in length, and these are traceable to the first feeding with eggs that took place 27 days before the death of the animal. These worms had developed to about four times the length of the largest that have been observed in experiments with the smaller laboratory animals.

From these experiments it is clear that the parasites behave in sheep and goats just as they do in rats, mice, guinea-pigs and rabbits, with the exception that after their return to the alimentary tract they are able to continue their development and approach the adult stage. These experiments also lend support to the common belief among parasitologists that the so-called *Ascaris ovis* occasionally found in sheep is merely the pig *Ascaris* in a strange host. It is of interest to

note that the specimens of *Ascaris ovis* whose measurements have been recorded are smaller than full grown specimens of *Ascaris lumbricoides*, and that fertile eggs appear never to have been seen. These are circumstances that are in accord with the results of our experiments and support the view that the sheep *Ascaris* is a parasite in the wrong host. Evidently the *Ascaris* of the pig is better adapted to existence in the sheep and goat than in rats, mice, guinea-pigs and rabbits. Apparently, however, it is unable to adapt itself sufficiently to reach the full measure of development attained in its usual host, the pig. In a scale of host adaptations we may therefore recognize three grades, rats, mice, guinea-pigs and rabbits in the lowest; sheep and goats in the intermediate grade, and pigs in the highest, with which we may also include human beings, if it be true that the *Ascaris* of man and of the pig are identical.

In our experiments on pigs we have found that the ingestion of *Ascaris* eggs by these animals is followed by the same series of phenomena that was observed in the experiments on other animals, including in some instances the occurrence of pneumonia. This has been noted in a previous paper (Ransom and Foster, 1917), and Stewart in one of his later articles (1918) has recorded the results of some experiments in which he observed the migration of *Ascaris* larvae through the lungs of pigs and the occurrence of pneumonia in these animals. Stewart, however, expressed himself as still unwilling to admit the development of *Ascaris* without an intermediate host. Owing to certain practical difficulties the experiments that we have thus far carried on with pigs as subjects have not been sufficiently controlled to exclude the possibility of the pigs themselves acting as their own intermediate hosts. That is, in all the cases in which we obtained intestinal infection with mature or nearly mature *Ascaris* following the feeding of the eggs to pigs, it is possible that the worms found had been reingested by the pigs after they had been passed out in the feces, continuation of their development beyond the lung stage having occurred only after such elimination and reingestion. So far, therefore, as our experiments on pigs are concerned, we cannot point to definite results disproving Stewart's views as to the necessity for an intermediate host. On the other hand no good evidence has yet been brought forth that *Ascaris* larvae after their migration through the lungs of an animal must necessarily pass out of the body and be reingested by the final host before they can develop to maturity.

There are certain important facts which have already been mentioned or alluded to by Lane (1917) and Low (1918) that are quite out of harmony with the hypothesis of the regular occurrence of the elimination and reingestion of *Ascaris* larvae as a necessity in the life

cycle. For example, the larvae after their migration through the lungs and elimination in the saliva or feces have very little resistance to unfavorable conditions, and though in moist media they can be kept alive for a time they quickly succumb to drying, a condition to which they are particularly liable to be exposed. The feeble resistance of the larvae after their passage through the lungs may be contrasted with the remarkable vitality of the eggs which have been kept alive for as long as five years, resist long periods of dryness, and are not killed by considerable periods of exposure to temperatures far below freezing. The egg stage is thus well adapted to withstand the hardships which the parasite must endure in its passage from one host to another, whereas the larvae that have passed through the lungs are not at all adapted to such an existence. On general principles it hardly seems probable that *Ascaris* could continue to exist if infection of the final host were brought about only by the ingestion of larvae which had already passed through the body of another animal and had been left exposed to the vicissitudes of the outer world in a feebly resistant condition.

As already stated, however, it may be admitted that infection in this way possibly sometimes occurs, though it has not yet been proved. On the other hand, the results of our experiments with the young lamb and the kid supply very strong proof of the correctness of the view that the final host becomes infected by swallowing properly incubated eggs and not by swallowing larvae that have passed through an intermediate host. In these experiments the very nature of the animals as well as their age practically excludes the possibility of infection through reingestion of larvae that had passed out of their bodies and dropped to the ground. In the light of the evidence from these experiments as well as that from the experiments with rats, mice, guinea-pigs, rabbits and pigs, and taking into consideration also the evidence which has been gathered by various investigators with reference to *Ascaris* infection of human beings, no other reasonable conclusion can be reached than that *Ascaris* has a direct life history without intermediate host, that infection occurs as a result of ingestion of the eggs, and that the larvae after migrating through the lungs return to the alimentary tract, settle down in the intestine if the animal is a suitable host and develop to maturity. It may be mentioned that very young pigs appear to be not only more susceptible to infection with *Ascaris*, but also more liable to develop pneumonia than other animals.

With reference to the production of pneumonia by *Ascaris* larvae it is of interest that Mosler (according to Leuckart, 1867) and Lutz (1888) observed lung symptoms in human beings a few days after the

ingestion of *Ascaris* eggs. In addition to the likelihood that *Ascaris* infection will be found to be responsible for certain lung troubles in human beings, particularly in children, it is quite likely that *Ascaris* has something to do with many of the cases of lung disease in pigs. Large numbers of young pigs suffer and die from lung affections the causes of which have never been satisfactorily explained. The symptoms shown by experimentally infected pigs at the time of the invasion of the lungs by the larvae are frequently exactly similar to those exhibited by pigs suffering from so-called "thumps," a popular name for a serious condition of very common occurrence among pigs, and it is accordingly not improbable that *Ascaris* is an important factor in the production of "thumps," especially when it is considered how very commonly *Ascaris* occurs as a parasite of pigs. Though we can not yet form a true estimate of the actual importance of *Ascaris* as a cause of lung disease it is evident that this parasite has capacities for harm not formerly suspected. Stewart's very interesting discovery of the migration of the larvae through the lungs has therefore not only added materially to our knowledge of the life history of *Ascaris*, but also by opening up a new line of investigation in pathology is likely to lead to a better understanding of the cause, prevention and treatment of certain diseases of the lungs.

The hatching of the eggs of *Ascaris* is an interesting question. It has been found by different investigators that when the eggs are swallowed hatching occurs in the small intestine. Hatching results not from any apparent digestion of the egg shell but from the active penetration of the shell by the contained embryo. Some writers have found that hatching will occur outside the body if the eggs are placed in certain solutions. We have been unable, however, to cause more than a very small percentage of the eggs to hatch outside the body in vitro. The factors which bring about the hatching of the eggs have not yet been determined. It is a noteworthy fact that if the eggs are injected beneath the skin of a guinea-pig they not only hatch, but that the larvae later appear in the lungs as they do following infection by way of the mouth, reaching a length of 0.5 mm. in seven days, and of 1.5 mm. in eleven days after the eggs are injected. Martin (1913) observed that the eggs of *Ascaris vitulorum* would hatch when introduced beneath the skin of a guinea-pig, but he did not follow the migrations of the larvae.

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OBSERVATIONS ON AND EXPERIMENTS WITH *CUTEREBRA TENEBROSA* COQUILLET¹

R. R. PARKER AND R. W. WELLS

The studies carried on by the Montana State Board of Entomology in the Powder River Valley in 1916 afforded the writers opportunity for chance observations on the rodent bot fly, *Cuterebra tenebrosa* Coquillet. Among over a thousand rodents captured for examination, as possible hosts of the Rocky Mountain spotted fever tick, *Dermacentor venustus* Banks, two were found, each infested with a single larva of this bot fly. The larvae were reared to adults and the following notes were made.

Record No. 1518, June 29. Found infesting breast of a pack rat (*Neotoma cinerea*). Air hole about three-sixteenths of an inch in diameter. Rat afterward died from trap injury and larva was squeezed out and placed on dirt. It immediately crawled in, the hole being left open. The fly emerged on August 25 after an interval of 47 days.²

Record No. 1526, July 19. Large bot larva found infesting grasshopper mouse (*Onychonomys leucogaster missouriensis*). It was embedded under the skin in front of the left hind leg. Larva emerged into bag in which mouse was placed for the ticks to crawl off. The adult emerged in Bozeman on Jan. 8, 1917, after an interval of 173 days.

On September 9, after returning to Bozeman, a living female of the fly was received in a box. The latter was left on the desk of the senior author for several days and when next examined was found to contain 186 eggs securely fastened to the pasteboard. They were not examined again until October 13, when the caps were removed from several eggs. Active larvae immediately crawled forth. This suggested the experimental infestation of rodents, and the experiments hereafter described were carried out. The larvae used were secured by removing the egg caps with a fine needle and gently assisting the larvae to make their escape. When the transfer was made to the mouth of the animal to be infested, the latter was made ready (prairie dogs were securely tied) and its mouth gently forced open and so held by a transverse pry. The cap of an egg was removed and the transfer made on the point of the needle as rapidly as possible and the animal released shortly thereafter.

1. Contribution from the Laboratory of the Montana State Board of Entomology, Bozeman, Montana.

2. This rat was also heavily infested with Siphonaptera and Anoplura as well as 45 larvae and nymphs of *Dermacentor venustus*.

Experiment 1. October 13. Four larvae transferred to the mouth of a prairie dog (*Cynomys ludovicianus*) and immediately disappeared. Three others were placed on a shaven spot on the neck, but it could not be seen that any effort was made to penetrate the skin. October 25, examination showed that 2 larvae had reached the subcutaneous tissue, one about at the middle of the left side (1), the other a little to the left of the middle beneath (2). Number 1 had punctured the skin and the larva was visible. It died about November 15, the abrasion healing quickly, leaving a hard lump that gradually disappeared. Number 2 was photographed on November 20 (Fig. 9) when the end could be seen very slightly protruding from the hole. It emerged during the following night and a photograph of the mature larva was made on November 21.

Experiment 2. This experiment was started with the hope that by using a considerable number of larvae, several being placed in the mouth at frequent intervals, that by subsequent dissection, the course of the larvae in the body might be traced. A prairie dog was again used as a host and 3 larvae were placed in its mouth on each of the following dates: October 27, 31, November 1, 2, 3, 4, 5, 6 and 7. A total of 27 larvae were used. On November 6 a small lump was noted on the belly, on November 7 this was slightly larger. On November 8 the host was killed and dissected, but no traces of the larvae were found either in the body cavity or tissues.

Experiment 3. Host, prairie dog. Three larvae were placed in its mouth on each of the following days, October 27 and 31, November 4 and 15. Larvae appeared under the skin as follows: (1) November 5 on right side, air hole noted on November 7; (2) November 16 on middle of neck dorsally; (3) November 17 slightly cephalad of (1) on right side; (4) November 17 slightly ventrad of (1) on right side; (5) November 17, on underside of left front thigh; (6) November 20, dorsal of middle of left side; (7) November 26 caudad and dorsad of left front leg.

On November 28, numbers 1, 4, 5 and 6 were dead, and numbers 2, 3 and 7 were still alive. Number 3 was removed, part of a cast skin coming with it. This was evidently the last molt and the chitin spangles had not yet become colored, the molt having apparently just taken place. The air hole was plugged at the time of removal. Numbers 4 and 7 emerged on December 13 and either crawled away or were destroyed by the dogs and were never recovered.

Experiment 4. Host, 13-striped ground squirrel (*Citellus tridecemlineatus pallidus*). Two larvae placed in its mouth on October 27 and 3 on November 21. Results negative.

Experiments 5, 6, 7 and 8. Hosts, Belgian hares. On November 8, 5, 4, 4 and 6, larvae were placed in the mouths of 4 Belgian hares, respectively. Results negative.

Experiment 9. Four larvae placed in the mouth of a prairie dog. December 14, 2 larvae appeared beneath the skin on the top of the fore shoulders. On January 8 one (1) was found to have emerged during the night, and on January 11 the second (2) was removed because of the condition of the host. This larva was located directly above the spine and was the only instance in which any of the prairie dogs gave evidence of being seriously affected by the presence of bot larvae. This dog had been used in one of the previous experiments. After removal the dog recovered rapidly. On January 8 number 1 was placed in a glass jar with 5 inches of dirt, on the 9th it had pupated about 2½ inches beneath the soil surface. Number 2 was placed in a similar jar on January 11, and after entering the ground voided considerable dark fluid from the anus. On January 12 it was still voiding similar excrementous matter. Pupation took place on January 14, but the insect died while in the pupal stage. On number 2, which was removed a little prematurely, the action of the larva in withdrawing and protruding the posterior spiracles indicated that the latter were protrusile before pupation. After pupation, however, they are

external as shown in figure 10. Number 1 had not emerged as an adult when the writers left Bozeman on March 25 for field work, and this record was never secured.

Experiment 10. On December 18 two larvae were placed in the mouth of a house mouse (*Mus musculus*). The host was dissected on December 20, but no larvae were found.

Naturally our curiosity was aroused as to where the eggs were deposited under natural conditions, the conditions under which the operculum was displaced, the manner in which the larva gained entrance to the host and course followed by the larvae in reaching the subcutaneous tissues. The following points are presented for what they are worth. The egg cap when dry requires sufficient force to remove it to make it seem doubtful if the larva within can be instrumental in forcing it off. When eggs are moistened with saliva a dark outlining band appears around the margin of the cap (Fig. 1). Of several eggs placed intact in preserving fluid the caps of several were later found to be off and the larvae slightly to almost wholly protruding. Whether the removal of the cap was due to the action of the larva upon being irritated by the fluid, which must have penetrated the egg slowly, or was due to some action of the fluid on the egg, is uncertain. At least, however, it seems evident that the caps must have become loosened very soon after being placed in the fluid, since the larvae were able to crawl part way out of the egg before being overcome. It is also of interest that though the eggs were deposited within a period of a few days following September 9, 1916, yet larvae in the eggs not used in the experiments were still active the following March, about six months later, and had not escaped from the eggs.

When larvae were removed from the egg and placed upon a surface they would immediately attach themselves by an apparently sucker-like organ (Fig. 4) at the posterior end of the body and sway the body back and forth. The same habit was observed when placed on the skin of an animal. It is the recollection of the writers that these minute larvae were able to move about to some extent, very much after the manner of an inch worm. Unfortunately, no notes were made on this point. It is distinctly recalled that the larvae looped themselves in the manner above suggested, and that in one instance a larva, removed from the egg and placed beside it on the box, was afterwards found several inches away. What may be the value of the ability to attach themselves is not evident. The eggs were firmly "glued" to the box in which they were laid, indicating that they are fastened to something when deposited under natural conditions. The ventral surface of the egg is broad on the posterior two-thirds and the surface of the egg on this area is somewhat sunken inward. If

this is a groove, it is certainly very wide and shallow if intended to be attached to a single hair. The eggs were all fastened by this surface. After the cap was removed a delicate membrane was frequently noted covering the opening; this had to be broken before the larvae could escape.

It is possible, if other related bot flies were kept alive when captured, that eggs and larvae might be secured in a similar manner and valuable and suggestive information gained concerning life histories and habits.

SUMMARY OF DATA RELATING TO LIFE HISTORY

1. Under natural conditions the larvae of *Cuterebra tenebrosa* were found infesting pack rats and grasshopper mice. Prairie dogs were infested under laboratory conditions, but negative results were secured with Belgian hares and 13-striped ground squirrels.

2. A female deposited 186 eggs within a period of several days. These eggs contained active larvae which were still alive after six months in the laboratory.

3. By mechanically transferring larvae from eggs to the mouths of prairie dogs infestation was secured. In three experiments with these animals (Exp. 2 excluded because the host was killed) 20 larvae were used, of which 11 reached the subcutaneous tissue, 5 died in this situation and 6 emerged as fully matured larvae. (One of these was dissected out just as it was completing the last molt.)

4. Evidence that the larvae had reached the subcutaneous tissue was found on the twelfth day in two instances, and within maximum limits of 9 and 10 days in two other experiments.

5. The length of time elapsing after the first apparent evidence of larvae under the skin and before the skin was punctured was about two days.

6. The period spent in the subcutaneous tissue was 17, 25, 26 and 27 days in the several cases observed.

7. The total period from infestation to the emergence of the fully developed larva was respectively 37, 38 and 47 days in three instances.

8. After emergence from the host the mature larva entered the ground and soon pupated a few inches below the surface.

9. The period between the emergence of the mature larvae from the host and that of the fly was 47 days (June to August) in one instance and 173 days in another (July to January 8).

10. Winter apparently may be passed in the pupal stage.

11. Prairie dogs seemingly experienced no serious effects from the presence of the larvae even when several were present simultaneously. Infested dogs sometimes seemed less active and often appeared to favor the part of the body infested. In one experiment in which the bot was located above the spine on the fore shoulders the most serious effects were noted. When larvae died in the dogs the air holes healed quickly leaving lumps that gradually disappeared.

EXPLANATION OF PLATE

Figure 1. Eggs moistened with saliva showing dark band demarking the operculum.

Fig. 2. Normal egg.

Fig. 3. Egg with cap removed.

Fig. 4. Larva just removed from egg. Note sucker-like extension posteriorly.

Fig. 5. Larva just after emergence from host.

Fig. 6. Anterior end of mature larva.

Fig. 7. Posterior end of mature larva.

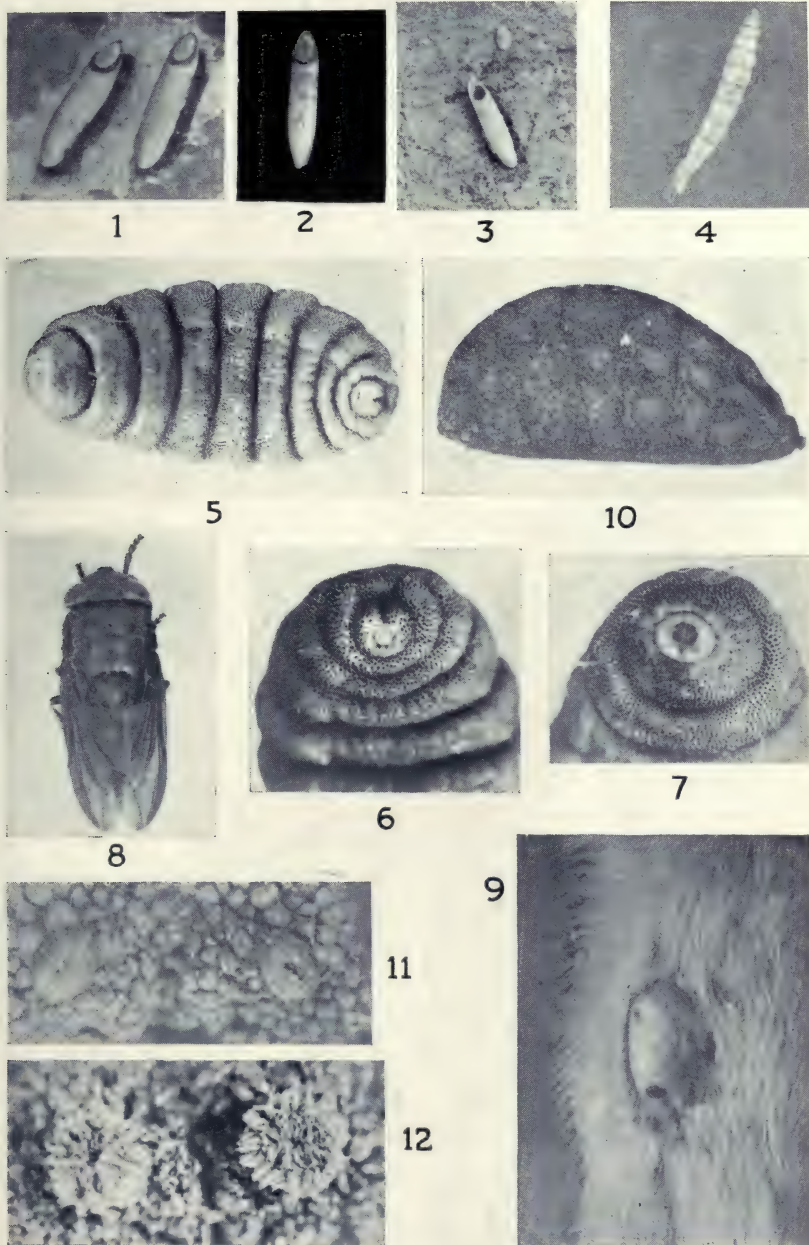
Fig. 8. Adult, female.

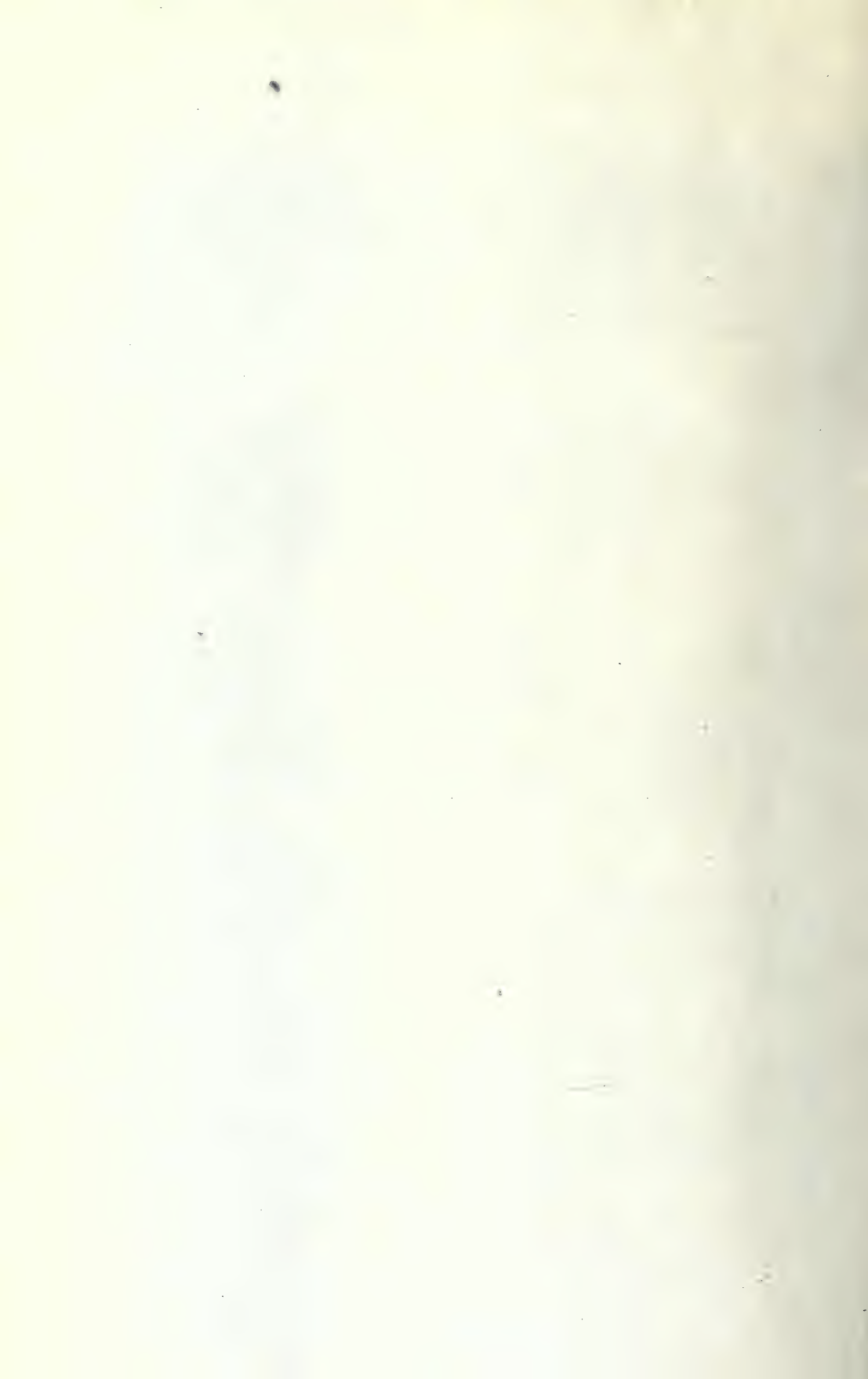
Fig. 9. Infested prairie dog just before emergence of larva.

Fig. 10. Puparium. Note spiracle posteriorly.

Fig. 11. Posterior spiracles of nearly mature larva with breathing apparatus retracted.

Fig. 12. Same, with breathing apparatus extruded.





ON THE DEVELOPMENT OF *ASCARIS LUMBRICOIDES* L.

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Development of uninjured fertilized eggs. Leuckart reported that in the hot summer months the eggs may develop and form an embryo in two weeks. In my experiments 15 to 18 days was the minimum length of time required, and in the great majority of the eggs 30 days was necessary, even in the summer months. Widely varying stages of development are found in a single culture. The optimum temperature is 28 to 34° C., room temperature being favorable to rapid development during the summer.

Lutz, Epstein and others have proposed various means of producing an embryo within the shell. In the present experiments a sample of fresh feces mixed with water was put into a large test tube (or small beaker) and the contents stirred with a glass rod and filtered through a sheet of gauze. The sediment remaining in the test tube is mixed again with water and the filtration repeated. After this process is repeated two or three times most of the eggs will be found in the filtered fluid, from which they are collected by centrifugation. By removing the rubber plug from the bottom of a short tube the egg masses that accumulate are easily transferred into Petri dishes or other shallow vessels. A small quantity of aseptic moistened sand is added and the dish covered with a piece of gauze and a glass cover to protect from flies, and at the same time to allow free admission of air. This culture method is quick, easy and safe. The eggs can easily be separated from the sand because of the difference in specific gravity. The dishes are put into an incubator at 28 to 34° C. in the winter or left at room temperature in the summer months and must be kept moist. Exposure to strong direct sunlight checks development, and if continued, will kill the eggs.

When development is finished the full grown, coiled embryo within the shell is very active, especially in a warm temperature. Such eggs as these are said to be mature and are able to infect the body of the host when taken into the alimentary canal. Experiments were begun in May, 1916, and have been continued up to the present time save for interruptions due to accident.

Non-infectiveness of Unripe Eggs

That eggs in the various stages of development short of maturity do not develop into the embryo in the alimentary canal of the host, but are evacuated in the feces is proved by the following experiments.

Experiment I. May 15, 1916. Seven guinea-pigs were fed with eggs from feces evacuated on the preceding day which showed little or no signs of beginning division. Three days later three of the pigs were killed. No eggs were found in the stomach and very few in the small intestine, but a good many in the cecum and large intestine. None showed any evidences of development.

May 25, 1916. Two more of the pigs were killed. No eggs were found in the alimentary canal.

June 22, 1916 (thirty-seven days after feeding). The remaining two guinea-pigs were killed and examined. No eggs whatever were found.

Experiment II. July 30, 1917, 3 p. m., a rat was fed with eggs 14 days old. July 31, 9 a. m., fecal examination showed small quantities of eggs. Three hours later larger quantities were found. August 1, fecal examination negative. August 3, eggs in abundance in the feces. August 4, 8 a. m., eggs in abundance in feces; animal killed at 9 a. m.; the organs, especially liver and lungs, examined for larvae but with negative results. Liver and lungs both normal.

Experiment III. August 31, 1917, a rat was fed with eggs 15 days old. September 1, 9 a. m., fecal examination showed a small number of eggs. September 2, 8 a. m., animal died. A few eggs were found at autopsy in the intestine, but no embryos.

Experiment IV. September 2, 1917, 2 p. m., a rat was fed with unripe eggs. September 3, 8 a. m., fecal examination revealed no eggs. September 4, 8 a. m., abundant eggs in feces; 12 m., animal killed and the organs, especially liver and lungs, examined for larvae, but with negative results.

None of the eggs used in the foregoing experiments had reached the mobile embryo stage.

Hatching Place of Eggs

Both stomach and intestine have been suggested by various authors as possible hatching places of the ripe eggs in the animal or human host. I have carried out both incubation and feeding experiments to determine the hatching place of the eggs. Ripe eggs placed in 0.8 per cent. salt solution and incubated at 38° C. for 5 days did not hatch, nor did ripe eggs hatch when incubated for 6 days at 37 to 38° C. in gastric juice. The experiment was repeated with a 1 per cent. solution of sodium bicarbonate as medium with the same results.

Experiment V. August 3, 10 a. m., a mouse was fed with ripe eggs 59 days old. August 4, 8 a. m., numerous eggs were found present on fecal examination. 1 p. m. (twenty-seven hours after feeding) the mouse was killed. The stomach contained numerous eggs, one half hatched-embryo, and three free embryos. The intestine contained three embryos and a good many eggs. Numerous eggs were found also in the cecum and large intestine.

Experiment VI. August 6, 4 p. m., two mice were fed with mature eggs 62 days old. August 7, 9 a. m. (seventeen hours after feeding) one mouse was killed. A small number of eggs were present in the stomach and small intestine, and numerous eggs in the cecum and large intestine, but no embryos. 11 a. m. (nineteen hours after feeding) the other mouse was killed. Eggs were found in the alimentary canal as in the first mouse, and in addition there were three embryos in the small intestine.

Experiment VII. August 3, 9 a. m., a guinea-pig was fed with mature eggs 59 days old. August 4, 8 a. m., eggs were found in the feces; 10 a. m. (twenty-five hours after feeding) the animal was killed. A few eggs were found in the

stomach and large intestine, but none in the small intestine. No embryos were found.

Experiment VIII. August 20, 2 p. m., ripe eggs 35 days old were given to a guinea-pig. August 21, 9 a. m. (nineteen hours after feeding) the animal was killed. A few eggs were found in the stomach and small intestine, some in the large intestine. No embryos were found.

Experiment IX. August 20, noon, ripe eggs 35 days old were fed to a rat with biscuits. The animal did not eat all of the biscuits until the next day. August 22, 10 a. m. (forty-six hours after feeding) the rat was killed. A few eggs were present in the stomach and lower part of small intestine. In the cecum there were three free dead embryos and a half hatched embryo, as well as numerous eggs, and in the upper part of the large intestine there were two free dead embryos and abundant eggs.

Experiment X. August 24, 4 p. m., three mice were fed with ripe eggs 35 days old. August 25, 9 a. m. (seventeen hours after feeding) one mouse was killed. A few eggs were found in the stomach and small intestine, and they were numerous in the cecum and large intestine, but there were no embryos; 12 noon (twenty hours after feeding) another of the mice was killed. The findings were similar to those in the first mouse. August 30, 11 a. m. (six days after feeding), the remaining mouse was killed. There were no eggs in the alimentary canal. The liver and lungs were normal.

Apparently the eggs used in Experiment X did not have sufficient life to develop. This was probably due to the fact that the culture from which the eggs was taken, together with other cultures, was exposed on August 24 to strong direct sunlight in an attempt to determine whether the sunlight would accelerate development. The contrary was thus proved to be true.

It is impossible to draw a conclusion from the six foregoing experiments with regard to the hatching place of the eggs, though the evidence, combined with that of previous investigators, favors the small intestine.

The average time of retention of eggs in the animals appears from our records of these experiments to be from 2 to 5 and usually 3 days. There were always some mature eggs among the greater number of immature eggs evacuated. It seems reasonable to assume that these apparently fully matured and healthy eggs were internally injured in such a way as to make further development impossible. It is clear that not all the mature eggs develop, especially in the case of a young culture. To obtain a heavy infection with *Ascaris* larvae it is necessary to use large quantities as completely developed as possible (40 days or more). The failure of many investigators to observe development of *Ascaris* within the animal host was perhaps due to lack of recognition of this point.

Migration of Larvae in the Body of Host

From the foregoing experiments, and from those of previous investigators, it seemed probable that the ripe eggs of *Ascaris* hatch in the

intestine of the animal within 12 to 19 hours, after which the larvae migrate into the other organs, particularly the liver and lungs. The following experiments demonstrate this latter point.

Experiment XI. June 23, 1916, two guinea-pigs were fed with mature eggs 40 days old. June 26 (seventy-seven hours after feeding) one pig was killed by mistake. Two small nematode larvae were found in the liver which afterwards proved to be young *Ascaris*. The remaining pig was killed June 30. It was impossible to find any nematode larvae in the liver. On opening the pleural cavity, however, the lungs were found to show heavy hemorrhage and to contain two small nematode larvae a little larger than those found in the liver in the preceding experiment.

Experiment XII. August 8, 1917, ripe eggs 66 days old were fed to a guinea-pig. Three days later the pig was killed. The liver, which was normal in color and in size, contained eight larvae. The lungs were normal.

Experiment XIII. August 3 and 4, a guinea-pig was fed with ripe eggs 59 and 60 days old, respectively. The animal was killed on August 7. The liver and lungs were externally normal. Eighteen larvae were found in the former.

Experiment XIV. August 10, a guinea-pig was fed with ripe eggs 66 days old. The pig was killed August 14. The liver was apparently normal, though somewhat lighter in color, and contained twelve larvae. The lungs were normal except for slight reddish spots and contained three of the worms.

Experiment XV. August 29, a guinea-pig was fed with mature eggs incubated in artificial gastric juice at 37 to 38° C. for six days, and also with some incubated in a 1 per cent. solution of sodium bicarbonate at the same temperature for five days. On September 3 the animal was killed. The liver was apparently normal, but faintly hemorrhagic and slightly lighter in color. It contained sixteen larvae. The lungs were somewhat spotted and contained nine of the worms.

Experiment XVI. July 24, a quantity of mature eggs 49 days old were given to a guinea-pig. Feeding with the eggs from the same lot was repeated on July 27. On August 1 the animal was killed. The surface of the lungs was covered with reddish or dark spots due to new and old hemorrhages, and fourteen specimens were obtained from it. Two larvae were found in the liver, one in the trachea and one in the small intestine (dead).

Experiment XVII. A guinea-pig was fed with a small quantity of feces containing ripe eggs and with mature eggs from a culture 56 days old on July 31, and again on August 1. The animal was killed on August 8. The lung surfaces were covered with hemorrhagic spots, and sixty-four specimens of larvae were collected from them. The other organs gave negative results.

Experiment XVIII. July 24, ripe eggs 49 days old were given to two young rats with food, which was not all eaten until the next day. The rats were put into separate cages on July 27, and rat A was fed with a large dose of ripe eggs. Rat B was killed on July 30. The liver only yielded specimens of the worms, two being contained in it. Rat A was killed August 2. The lungs were somewhat spotted with hemorrhages and contained eight worms. The other organs were negative.

The foregoing experiments indicate that the completely matured eggs hatch in the intestine, whence the larvae migrate to the liver, lungs, and trachea, returning finally through the pharynx to the intestines. The liver is apparently not affected by the larvae, but the lungs usually show considerable hemorrhage.

The Fate of the Larvae in the Body of the Feeding Animal

The larvae finally reaching the intestine in these feeding animals are apparently not able to develop into adult form there, being voided with the feces within a few days, presumably because the animals are not the normal host of the parasite. Hence, it seemed reasonable to suppose that completely developed larvae from the lungs or trachea of feeding animals, when ingested by the human being, will grow into adult form in the human intestine. To determine this point 35 specimens of larvae from the lungs of a guinea-pig were taken by the writer on August 8. The details of the experiment will be described later.

The following several experiments were made to determine the period during which the larvae appear in the various organs of the feeding animals.

Experiment XIX. October 11, 1917, two guinea-pigs were fed with ripe eggs, 42 days old. Twenty-four hours later guinea-pig A was killed. Embryos were not found in any part of the alimentary canal or even in the abdominal cavity, and only a few in the stomach and intestine. Guinea-pig B was killed on October 19. Six large larvae (2 mm. in length) were found in the left lung, which was severely hemorrhaged, and three in the trachea.

Experiment XX. November 27, six guinea-pigs were fed with ripe eggs, 68 days old. Guinea-pig A was killed on the next day, and two living larvae were found in the large intestine, but none in other organs. Animal C was found dead on December 3. The liver was slightly infected, but the lungs were heavily hemorrhaged and consolidated. The upper part of the trachea contained two larvae. Animal D was found dead on December 5. The liver was normal. The lungs were dark brown, hemorrhagic, and contained numerous larvae. A few larvae were found in the trachea. Animal E was killed on December 9. The lungs were extremely hemorrhagic, consolidated and dark brown in color, but contained no larvae. Two larvae were found in the trachea and five dead ones in fecal pellets from the rectum. Animal F was killed on December 10. Lungs: As in the preceding animal. One specimen was found in the lung, and three in the large intestine, but none in the trachea.

Experiment XXI. December 12, two guinea-pigs were fed with ripe eggs, 85 days old. Animal A was found dead on December 14. The liver was heavily infected with the larvae. The abdominal cavity was free from the larvae. Guinea-pig B was killed on December 15. The liver was severely infected. The lungs were slightly hemorrhagic and contained a few larvae.

Experiment XXII. October 29, ripe eggs, 60 days old, were given to two guinea-pigs, one of which was found dead on November 1 (animal A). The liver was severely infected with larvae. The other organs were free. Animal B was killed on November 7. The liver was normal. The lungs were spotted with dark brown color, and the anterior lobe of the left lung was consolidated. Numerous larvae were found in the lungs and trachea, one dead specimen in the small intestine, and four living and one dead larva in the large intestine. The trachea contained three specimens in the upper portion, twenty-four in the middle portion and fifty-one in the lower. Fifty of these completely developed living specimens were later ingested by the writer. They varied from 1.23 to 1.62 mm. in length.

Experiment XXIII. November 10, two guinea-pigs were fed with ripe eggs, 54 days old (to A) and 72 days old (to B). B was killed November 14. The liver was heavily infected, and the lungs slightly hemorrhagic and apparently consolidated. Three specimens of larvae were found in the heart. Animal A was found dead on November 25. The outer surface of the lungs was light red and the inner surface dark red in color. They were hemorrhagic and consolidated. Three specimens were present in the left lung, six in the trachea, which contained a small quantity of bloody mucus, and two dead larvae in the large intestine.

Experiment XXIV. November 15, a guinea-pig (A) was fed with ripe eggs, 57 days old, cultivated in a 0.5 per cent. solution of hydrochloric acid, and another (B) with eggs of the same age cultivated in a 0.2 per cent. solution. A was found dead on the 20th. The liver was infected with larvae, and the lungs were hemorrhagic, consolidated and contaminated with numerous larvae. Animal B was found dead on November 22. The liver was slightly infected. The lungs were severely hemorrhaged and apparently consolidated. Abundant larvae of small size were found in the organ. The trachea contained a small quantity of bloody mucus in which numerous larvae were found.

Experiment XXV. November 17, two guinea-pigs were fed with fully matured eggs, 59 days old cultivated in a 0.1 per cent. solution of carbonic acid. A was found dead in the morning of the 22d. The larvae were found in the liver and lungs, and the latter was dark brown in color from heavy hemorrhage. The trachea contained a small quantity of bloody mucus in which three small larvae were present. Animal B was found dead November 26. The liver was free from the larvae. The lungs were dark brown from hemorrhage, consolidated, and contained numerous larvae of various sizes. There were also larvae in the trachea.

Experiment XXVI. November 8, guinea-pig A was fed with ripe eggs, 58 days old, and B with ripe eggs cultivated in a 0.3 per cent. solution of carbonic acid for seventy days. C received mature eggs 70 days old. Animal A was killed on the 13th. The liver and lungs were infected, the trachea free. The lungs contained light and dark red hemorrhagic spots. Two larvae were found in the heart. B was found dead on November 17. The lungs were dark brown with light red margin and were somewhat infected with larvae. Numerous larvae were found in the trachea and six in the large intestine. C was found dead on November 19, and eight large specimens (1.75 to 1.91 mm. long) were found in fecal pellets from the rectum.

Experiment XXVII. November 16, two guinea-pigs were fed with eggs 58 days old. Animal A was found dead on the 24th. The liver contained a few worms. The lungs showed heavy hemorrhage and contained numerous specimens of the worm. The trachea contained some blood and two larvae. B was killed on November 26. The lungs showed severe hemorrhage, were consolidated, and contained a few larvae. The trachea and large intestine also contained a few.

Experiment XXVIII. October 20, two guinea-pigs were fed with eggs 51 days old. Animal A was lost; B was found dead on the 29th. The liver was free. The lungs were somewhat hemorrhagic and contained numerous larvae.

Experiment XXIX. October 20, a guinea-pig was fed with ripe eggs cultivated in a 0.1 per cent. solution of carbonic acid for 51 days. It was found dead on the 29th. The lungs were severely hemorrhaged and consolidated, and large larvae were abundant in the lungs and trachea. Three living larvae were also found in the large intestine.

Summary of Experiments XIX to XXIX

The larvae first appear in the liver on the second day (about 44 hours after feeding), and they are present until the sixth or seventh day, being most abundant on the third, fourth and fifth days, and disappearing after the eighth day.

As Experiments XXI and XXIII show, the larvae are first found in the lungs on the third or fourth day, and a few persist even to the fourteenth or fifteenth day after feeding. They are most abundant from the sixth to the eighth day.

In the trachea the larvae appear first on the fifth or six day and persist as long as they are present in the lungs, being most abundant on the eighth and ninth days. From the trachea they migrate into the mouth and thence down the alimentary tract of the host. The appearance of larvae in the alimentary tract begins after the eighth day. They are rarely found in the esophagus, stomach and small intestine, apparently passing through rapidly, but accumulate in the cecum and large intestine, where some of them are found dead. All are sooner or later voided in the feces.

Severely infected lungs are almost always hemorrhagic, consolidated, and dark brown in color. Epistaxis was not encountered in the animals of these experiments, but Stewart has reported its frequent occurrence in the infected rat.

Experiments on Other Mammals

Further experiments with regard to migration were made in other mammals and in the human body. For this purpose the rabbit, cat and monkey were used.

Experiment XXX. January 22, 1918, two monkeys were fed with large quantities of ripe eggs 76 and 87 days old. A was again fed with a large dose of eggs, 91 days old, on the 26th. B was found dead on the 29th, and *Ascaris* larvae were found in the lungs. Monkey A was killed on February 1. No larvae were found in the liver. The lungs were heavily infected. Two larvae were found in the trachea, large quantities in the stomach, and a few in the small intestine. The lungs were slightly hemorrhagic and consolidated.

Experiment XXXI. March 9, a cat was fed with eggs 131 days old. March 11, there was another feeding from eggs of the same lot. The animal was killed on the 18th. The lungs were slightly hemorrhagic and infected.

Experiment XXXII. A rabbit was thrice fed with *Ascaris* eggs, on December 29, 1917; January 21, and March 9, 1918. On March 19 the rabbit was killed. The lungs were slightly hemorrhagic and infected with numerous larvae. A few larvae were present in the trachea and small intestine.

From these experiments it is concluded that the migration of *Ascaris* larvae is definite in any feeding animal in which the larvae are alive. It was therefore to be supposed that they follow the same course in the human body as in the feeding animals. Personal experiments later confirmed this belief.

Experiments on Immunity

The next point to determine was whether or not the infected animal requires any protection against a second infection with *Ascaris* larvae.

Experiment XXXII. The intervals between the first and third and between the second and third feedings were seventy and forty-seven days, respectively. The rabbit was killed on the tenth day after the feeding, and its lungs, trachea and small intestine were infected with the larvae.

Experiment XXXIII. January 21, a guinea-pig was fed with ripe eggs 81 days old. March 7, it was again fed with eggs 91 days old. The animal was killed on March 14, seven days after the last feeding. The liver and lungs were slightly infected and the latter somewhat hemorrhagic.

The animals in these two experiments, having recovered from the earlier infections, apparently had no protection against subsequent ones.

Experiment XXXIV. January 23, a guinea-pig was fed with eggs 78 days old; March 7 the feeding was repeated with eggs 129 days old. It was killed on March 18. No larvae were found.

Experiment XXXV. Two successive feedings of eggs 129 days old were given on March 7 and March 9. When it was killed on the 27th, eight days after the last feeding, it was found to be uninfected.

The results of the two last experiments might be ascribed either to the presence of an immunity acquired by the previous infection or to the inability of the eggs used in the second feeding to develop, the eggs having been in dry condition for some days. Stewart has reported a case in which the rat was immunized by one infection with *Ascaris* larvae. Prof. Fujinami reported the immunization of a horse by one heavy infection with *Sch. japonicum*.

The Migrating Power of the Larvae

Experiment XXXVI. On March 16 *Ascaris* larvae from the liver of a guinea-pig killed sixty-seven hours after feeding were injected into the abdominal cavity of two guinea-pigs. Guinea-pig A was killed March 23. The lungs were slightly hemorrhagic and infected. B was killed on the following day. The condition of the lungs was the same as in A. The small portions of the middle lobes on both sides were dark gray in color and evidently consolidated.

It was not determined in this experiment by what route the injected larvae migrate into the lungs — by the blood vessel after penetrating the liver, or by penetrating the lungs from the surface after passing through the diaphragm. The experiment gives clear evidence, however, of the power of the larvae to pass through the tissues of the host. Nor it is precisely determined by what route the larvae migrate from the intestine to the liver and from the liver to the lungs of the experimental animal. It is thought that there are three routes by

which the larvae may pass from the intestine to the liver: (1) by the blood vessels distributed on the wall of the alimentary canal; (2) by the common bile duct, and (3) by penetrating through the intestinal wall and through the surface of the liver. The route last mentioned seems most probable from Experiment XXXVI, but none of my attempts to determine this point were successful. Stewart supposed that the larvae penetrating the alimentary wall are carried into the liver by one of two routes, by the mesenteric venules or by the bile duct, he did not determine which.

Between liver and lungs two courses of migration are supposed to exist, the blood vessel and the body cavity. Experiments XXIII and XXVI seem to favor the former, but Experiment XXXVI suggests that the latter course is possible. Further investigations on this subject are being carried out.

Human Experiments with Ascaris Larvae

On August 8, 1917, the writer swallowed 35 larvae of various sizes taken from the lungs of a guinea-pig killed on the seventh and eighth days, respectively, after two feedings. Examinations of the feces for the eggs on September 1, 8, 13, 17, October 19 and November 8 were negative, probably because the larvae were not completely developed. Hence larger larvae from the lungs or trachea were used in the second experiment. October 19, 1917, five specimens (2 mm. long) were swallowed. These were taken from the lungs of the guinea-pig killed on the eighth day after feeding. On November 7, 50 specimens were ingested, 1.61 mm. long and coming from the trachea of a guinea-pig killed on the ninth day after feeding. Examination of feces for the eggs on December 8, 18, 29 and January 8 were negative. On January 21, 75 days after the last feeding, however, numerous eggs were present in the feces. Since that time it has often been possible to find the eggs in the feces, and since there is no other explanation of the infection it is reasonably certain that it is the result of these experiments.

Morphological Changes in the Larvae During Development

The detailed study of this part of the subject is not yet complete. The embryo in a mature egg coils its body once or twice within the shell and is actively mobile. The larva just emerged from the egg-shell is slender, cylindrical in shape, with a rounded anterior and pointed posterior extremity, the latter conical and slightly turned

toward the dorsal side. The anus is ventrally situated at the base of the conical portion of the posterior extremity. A small portion of each extremity is homogeneous and transparent in appearance, the anterior being a little larger than the posterior end. The greater portion of the body is internally granulated, indicating the intestine and the lower part of the esophagus.

The larvae in the intestine, liver, and lungs are also very active and are always coiled, like the adult form. It is perhaps a characteristic movement of the species. Embryos measured varied in size from 0.23 to 0.27 mm. in length and from 0.013 to 0.017 mm. in breadth.

The larvae in the liver as they mature become differentiated in organization. Three lip-like processes on the front of the body become more visible. The esophagus, which occupies one-third of the intestinal tract, takes a definite form, its posterior end being slightly swollen, its wall very thick, and the lumen very thin but clearly visible. Near the middle of the esophagus there is presently recognizable a group of cells which should be the foundation of the nervous ring of the adult form. The intestine is also differentiated in that the thick wall and the thin lumen are distinguishable. It runs straight along the median line of the body and occupies two-thirds of the length of the digestive canal. A cellular layer of the body wall may be easily distinguished from that of the intestinal wall by a thin space which is regarded as the body cavity. The genital area appears as a distinct cell in the ventral side at about one-third of the body length from the posterior end.

Table 1 shows the measurements of various parts of the body in living specimens.

TABLE 1.—DATA FROM LIVING SPECIMENS

	Total Length mm.	Maximum Breadth mm.	Length of Esophagus mm.	Distance of Anus from Posterior End mm.
1	0.364	0.021	0.1	0.25
2	0.417	0.02	0.117	0.026
3	0.435	0.025	0.107	0.022

In the lungs the larvae become thrice or four times as large as those in the liver, but there is no remarkable difference in structure. The differentiation of organs become daily more apparent. They vary in size according to the date of infection and are found in the blood vessels, alveoli and bronchi. The intestine of the fully grown larva in the lungs is yellow or light brown in color, perhaps due to food particles.

Table 2 shows the measurements of specimens mounted in potassium acetate or in glycerin.

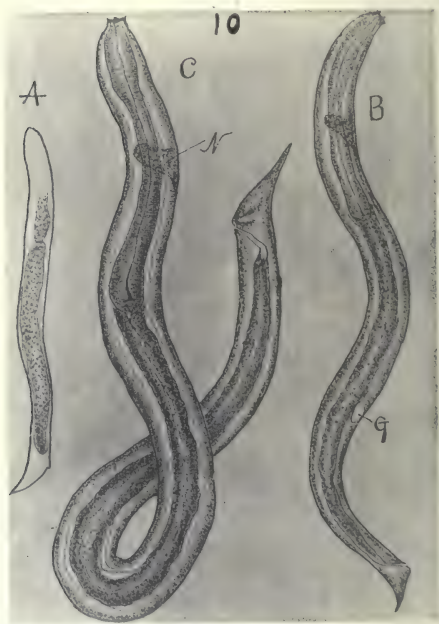
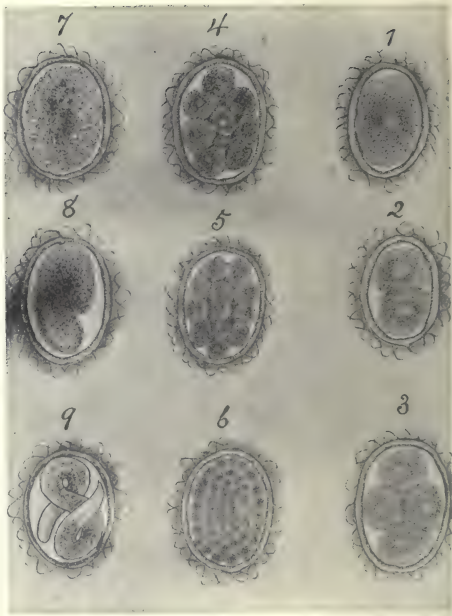
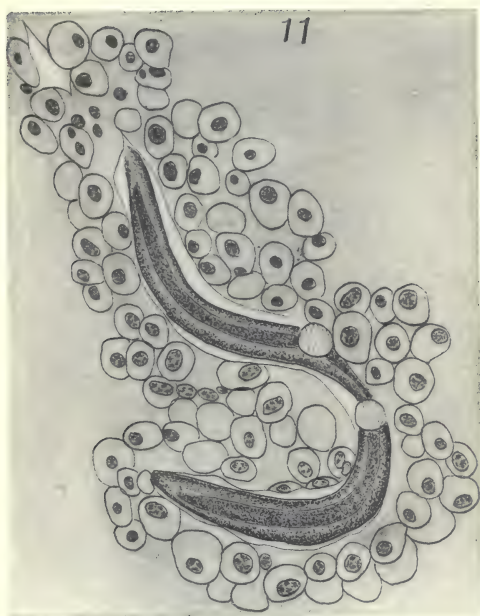


TABLE 2.—SPECIMENS MOUNTED IN POTASS. ACETATE OR GLYCERINE

	Body Length	Maximum Breadth	Length of Esophagus	Distance of Anus from Posterior End
	mm.	mm.	mm.	mm.
1	1.90	0.056	0.28	0.062
2	1.70	0.052	0.26	0.061
3	1.62	0.053	0.26	0.06
4	1.45	0.051	0.25	0.06
5	1.23	0.047	0.23	0.055
6	1.014	0.042	0.2	0.053
7	1.857	0.042		
8	0.857	0.034	0.178	0.050
9	0.846	0.039	0.228	0.050
10	0.666	0.038		
11	0.657	0.030	0.214	0.052
12	0.571	0.028	0.125	0.035
13	0.385	0.028	0.128	0.035
14	0.357	0.028	0.072	0.032
15	0.300	0.021	0.064	0.025

The larvae in the trachea and alimentary canal are similar to the fully developed ones in the lungs.

It is my pleasant duty to express my sincere thanks to Prof. A. Sato, Director of the Osaka Medical Academy, for his kind advice and help during the course of this work. I am also indebted to several other persons who have given me cordial assistance in the investigation.

EXPLANATION OF PLATE

Figs. 1-9. Stages of development of *Ascaris* egg. $\times 300$.

Fig. 10. Embryo just hatched in the intestine of guinea-pig. $\times 233$.

Fig. 11. Larva from the liver of guinea-pig. $\times 233$.

Fig. 12. Larva from the lung of guinea-pig. $\times 233$.

Fig. 13. Larva in the liver of guinea-pig. $\times 500$.

Fig. 14. Larva in the lung of guinea-pig. $\times 300$.

Abbreviations: g, genital cell; n, nerve ring; o, dust; v, alveola.

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ON A SPECIES OF HEDRURIS OCCURRING COMMONLY
IN THE WESTERN NEWT, *NOTOPHTHALMUS*
TOROSUS *

ASA C. CHANDLER

It has recently been pointed out by Ward and Magath (1916) that the nematode fauna of North American freshwater fishes is almost unknown. From a survey of the literature this state of affairs appears to be almost if not quite as true of the nematodes of other cold-blooded vertebrates in North America.

While making stomach examinations of the Western Newt or water-dog, *Notophthalmus torosus*, the writer found a large percent of specimens captured in the vicinity of Corvallis, Oregon, infested by a nematode which was evidently a species of Hedruris. The structure and ecology of this worm are so unique and proved so interesting and the references to it in the literature are either so slight or so difficult of access, that it seemed worth while to draw the attention of American helminthologists to it. Hitherto the members of the genus Hedruris have been looked upon as rare worms, and many helminthologists are no doubt unfamiliar with them.

According to Perrier (1871), whose account of *Hedruris armata* is by far the most thorough that can be found in literature of any species of Hedruris, these peculiar worms were first known to Rudolphi, but Perrier says: ". . . neanmoins leur caractères si particuliers lui ont complètement échappé, et il nous semble assez difficile de reconnaître, comme le fait Schneider (Monogr. der Nemat., p. 107), l'*Hedruris androphora* dans l'*Ascaris acuminata* du savant helminthologiste." Nitzsch (1821) described the first known species, *H. androphora*, in a readily recognizable manner, and proposed its separation from the genus *Ascaris* into a new genus Hedruris. The first and only good description of *H. androphora*, the type species, is given by Schneider (1866). This species is found in various European amphibians and has been recorded from *Bufo calamita*, *Bombinator igneus*, *Triton cristatus*, *Lissotriton punctatus* and *Proteus anguineus*, always attached to the mucous membrane of the stomach.

Leidy (1851), apparently unaware of the existence of *H. androphora*, described a worm, evidently a Hedruris, which he found in the stomach and commencement of the small intestine of *Clemmys guttata*, and named it *Synplecta pendula*. Baird (1858) very inadequately

* Contribution from the Zoological Laboratory, Oregon Agricultural College.

described a Hedruris from the axolotl, "*Siredon mexicanus*"; the larval form of *Ambystoma tigrinum*, giving it the name *Hedruris siredonis*. Perrier (1871) described another species, *H. armata*, from the back part of the mouth cavity of *Chrysemys picta*. His description is, as already remarked, by far the most thorough of any which can be found in the literature. From the time of Perrier's paper to the present, no further research on this unique genus has been done, and very few references to it, even of the most casual kind, can be found.

The following is a diagnosis of the genus based on the descriptions of other authors and on the writer's observations on the species found in *Notophthalmus torosus*:

Cylindrical whitish worms, tapering from a fairly robust posterior end to a slender anterior end; surface of body more or less finely striated; mouth provided with two pairs of lips, a median and a lateral (Figs. 2 and 3); median lips (Fig. 2) thin, chitinized, concave on the upper surface, and attached to the worm only by the middle portion of the base; lateral lips (Fig. 3) enclosed by median ones, thick with elaborate chitinous skeleton, and united by a chitinous commissure; esophagus long, slender, muscular, crowned by a festooned chitinous ring and penetrating valve-like into the intestine, its lumen four-cornered, not three-cornered; intestine straight, cylindrical, terminating in a chitinous rectum in the female.

Female attaching itself to mucous membrane of host by means of posterior portion of body, which can be invaginated sucker-like and has attached to it a chitinized hook resembling the claw of a cat, the latter being used to hook into the mucous membrane of the host and to draw it up into the interior of the invaginated portion of the body (Fig. 5); female reproductive system double, with dilations of the oviducts which act as seminal vesicles (Fig. 7); vulva posterior, shortly anterior to the anus; eggs (Figs. 8 and 9) oval, provided with terminal opercula as in Trichuridae, and containing developed embryos when deposited.

Male smaller and slenderer than female, always rolled about his mate by about three spiral turns of the body; surface of body, anterior to anus, in contact with female on inner surface of spiral coil is provided with 15 to 20 rows of quadrangular tubercles, reminding one forcibly, both in appearance and function, of the elevations on certain kinds of non-skid tires (Fig. 6); spicules short, equal, crescent-shaped, with or without a small gubernaculum; one pre-anal and at least six postanal papillae.

Habitat of species so far known always stomach or back of mouth cavity of amphibians and pond turtles.

In many of the respects named above, Hedruris is absolutely unique among nematodes, and it is evidently a very highly specialized form. There can be no doubt, however, that it finds its nearest relatives among the Spiruroidea, and should be considered representative of an aberrant family belonging to this group as suggested by Railliet (1916). Perhaps the forms which approach nearest to it are the species of the genus Habronema (cf. figures by Ransom, 1913). The form and arrangement of the lips and the armature of the mouth; the chitinous crown for the esophagus (Figs. 2 and 3) and the general

form of this organ; the form of the vagina, with a muscular mass surrounding its distal end (Fig. 5); the dorsal curvature of the tail of the female (Figs. 1 and 5); the chitinized rectum; the form, but not arrangement, of the male caudal papillae; are all strikingly similar to conditions found in *Habronema*. The cervical papillae of *Habronema* are also remarkably suggestive of the tactile spines described and figured by Perrier for *Hedruris armata*. Furthermore, the ventral surface of the body of the male of *Habronema*, anterior to the anus, is remarkably like that in *Hedruris*. Similar modifications occur in *Arduenna* and other spiruroids. On the other hand, *Hedruris* is less specialized than other spiruroids in the short, equal spicules, and more specialized in the development of the caudal adhering apparatus of the female, in the presence of distinct seminal vesicles, in the relation of the sexes, and also in the degree of specialization of the lips.

As already shown, four species of *Hedruris* have already been described, and considerable confusion exists as to the status and relationships of the species. In an endeavor to ascertain the identity of the species found by the writer in *Notophthalmus torosus* a careful comparison of the published descriptions of the species has been made and certain conclusions have been reached, though the incompleteness of the descriptions makes it necessary to assume more than is desirable.

The first described species, and therefore the type of the genus, is *H. androphora* Nitzsch. It may be defined as a small species, not exceeding 10 mm. in length in the female and 8 mm. in the male, within distinctly striated cuticula, and with mammillated eggs; it occurs as a stomach parasite of various European amphibians. The only other well-described species is that of Perrier, *H. armata*, which differs from *H. androphora* in its larger size (♀ 23 mm., ♂ 20 mm. long), in its distinctly striated cuticula in both sexes, in its non-mammillated eggs, and in the presence of tactile cervical spines; it occurs as a pharyngeal parasite of the American *Chrysemys picta*, having been obtained from a specimen in the menagerie of the Paris Museum of Natural History.

Perrier, when describing his species, was evidently unaware of the species described by Leidy twenty years before under the name *Synplecta pendula*. So far as Leidy's description goes, it appears to harmonize perfectly with that of Perrier. The size of Leidy's specimens (12.5 mm. to 23 mm. for the female and 8 to 10 mm. for the male), the marked striation of the body, the number of caudal papillae of the male, and the lack of any mention of the eggs being mammillated on the sides, all ally this species with *H. armata* and not with *H. androphora*. Leidy does not mention cervical spines in his species, but one would hardly look for such a minute detail in such a superficial description. Leidy's specimens were taken from the stomach and commence-

ment of the small intestine of *Clemmys guttata*, which, like *Chrysemys picta*, is a common pond turtle in Eastern United States. It appears very likely, therefore, that *H. armata* and *H. pendula* are synonyms, in which case the latter name would have to stand. Stiles and Hassall (1894) refer to specimens of Hedruris in Leidy's collection in the University of Pennsylvania taken from "*Ambystoma mexicanum*" and "*Nanemys guttata*" as *H. androphora*. Baird's *H. siredonis* is so meagerly described that its identity can only be inferred from circumstantial evidence. His description is based on a single female specimen, probably immature, taken from an axolotl from Mexico. The size, 13 mm., and marked striation of the body, the only two characteristics of specific value mentioned, are evidence that it is not *H. androphora*. There is nothing except the host to distinguish it from *H. armata* or *H. pendula*, and it would naturally be included with them were it not for the fact that the specimens found by the writer in the western newt evidently represent a species distinct from either *androphora* or *armata*, and the probability is in favor of Baird's specimen being identical with the writer's species. So far as it goes, Baird's description agrees with the species found in *Notophthalmus torosus*, and its geographic occurrence and host both point to a probable identity of the two worms. Provisionally, therefore, the writer will refer to his specimens as *H. siredonis* Baird.

As found in *Notophthalmus torosus*, the last species appears to be more or less intermediate between *H. androphora* and *H. armata*. It is intermediate in size, the full grown females (Fig. 1) being 16 mm. to 17 mm. long, with a maximum width of from 0.5 mm. to 0.6 mm., while the males are 8 mm. to 10 mm. long by 0.23 mm. wide. The body of the female is very coarsely striated; the striations are about 50 μ apart, and each in turn is marked by from 12 to 15 exceedingly fine striations (Fig. 5). The body of the male, on the other hand, is very indistinctly striated, the striations being so light that when the body is curved they cannot be seen at all on the greater curvature. Male specimens cleared in carbolic acid show no evidence whatever of striation, whereas even the fine substriations of the female can be observed clearly. Often the striation of the male seems to be entirely missing, at least on parts of the body. This indistinctness of striation in the male is in contrast with the condition in *H. armata*, in which Perrier says the male is striated evenly from head to tail. There is no evidence whatever of cervical spines in *H. siredonis*. The male possesses a small gubernaculum in addition to the spicules, and has altogether ten pairs of postanal papillae, seven near the midventral line, two placed more laterally near the top of the tail, and one just behind the anus (Fig. 6). The eggs (Figs. 8 and 9) resemble those of

H. androphora in their operculum, and also in the mammillated surface, a feature which is conspicuously absent from *H. armata*. The lips (Figs. 2 and 3), both median and lateral, are shaped a little differently in *H. siredonis* than in *H. armata*.

In other characteristics of structure and anatomy *H. siredonis* agrees with Perrier's description of *H. armata* (Fig. 7). The reproductive system is built on the same plan, but the ovaries with their radially arranged eggs are longer, and the seminal vesicles are more distinctly marked off. The branches of the uterus also are larger and contain a greater number of eggs than is the case with *H. armata*, according to Perrier's figure. Hedruris is the only nematode known to the writer in which a completely distinct seminal vesicle is present. Hall describes a slight dilation of the anterior ends of the uteri of *Dermatoxys veligera* as a seminal vesicle; in this genus there is also a slender oviduct connecting ovary and uterus.

To sum up, the species of Hedruris may be characterized as follows:

1. Size small, ♀ not exceeding 10 mm. in length; indistinctly striated if at all; eggs mammillated; in stomach of European amphibians.

H. androphora Nitzsch.

2. Size medium, ♀ 16 mm. to 17 mm. long, distinctly striated; ♂ indistinctly or not striated; eggs mammillated; in stomach of *Notophthalmus torosus* and axolotl.

H. siredonis Baird.

3. Size large, ♀ 23 mm. long; both sexes distinctly striated; a pair of cervical tactile spines; eggs not mammillated; in pharynx of *Chrysemys picta* (probably identical with *H. pendula* Leidy from stomach of *Clemmys guttata*).

H. armata Perrier.

Unlike any previously described Hedruris, *H. siredonis* is an abundant parasite, at least in the vicinity of Corvallis. The majority of all specimens of *Notophthalmus torosus* examined are infested, frequently by a few worms, but sometimes by 20 to 25 pairs. Very often the females exceed the males in number, i. e., there are frequently females unaccompanied by males. Unattached males have never been found. Since it is always adult ripe females which are unaccompanied, it is to be presumed that the males die sooner than the females, and pass out of the digestive tract of the host. Nitzsch, however, states that he has found young females of *H. androphora* unaccompanied, assuming that they were too delicate to support the males.

That the worms when present in large numbers have an injurious effect on their hosts is evident. The mucous membrane of the stomach around the places where the worms adhere is often considerably swollen, and the point of attachment of the worms can sometimes be seen from the outer surface of the stomach. Perrier demonstrated

that the caudal claw of the female worm is perforated and connected with a gland, thus imitating closely the fang of a solenoglyph snake, and, as suggested by Perrier, the probable function of the secretion of the gland is to irritate the tissues of the host sufficiently to cause a swelling, the latter making the attachment of the worm the more secure. On several occasions the writer has observed that heavily infested newts have been undersized and thin, and contain little or no food in the stomach.

H. siredonis, as observed in life, is an extremely interesting animal. The invaginated posterior end of the female is seldom entirely everted, usually only far enough to throw the claw into such a position that it can seize a surface in contact with it and draw it back into the invagination. The worms are in general sluggish in movement, and appear to be incapable of the rapid swimming in which many nematodes indulge. The male worms, when slid off from the females, can only partially uncoil, and the permanent nature of the coils is demonstrated by the fact that when freed males are immersed in hot formalin the posterior end invariably coils in such a way as to leave a tube corresponding to the diameter of the female worm.

The life history of *Hedruris* is as yet unknown, outside of the fact, stated by Perrier, that the embryos escape from the eggs upon slight pressure, or sometimes spontaneously when in fresh water, probably due to osmosis. By analogy with other spiruroid worms, and from the fact that very small individuals are never found in the newts, it is probable that the eggs or embryos are swallowed by an intermediate host in which the early stages of development are passed, and that infection of the definitive host is brought about by feeding on the intermediate host.

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EXPLANATION OF PLATE

Fig. 1.—Adult female *Hedruris siredonis*. $\times 11$.

Fig. 2.—Anterior end of body of δ *H. siredonis* showing median lip. Note chitinous commissure connecting lateral lips, and chitinous crown of oesophagus. $\times 132$.

Fig. 3.—Anterior end of young φ , showing lateral lip. Note chitinous crown of esophagus; comparison of Figures 2 and 3 will show that the lumen of the oesophagus is four-cornered. Note also excretory pore and nerve ring. $\times 110$.

Fig. 4.—Male and female *H. siredonis* in situ. $\times 11$.

Fig. 5.—Posterior end of adult φ *H. siredonis*, showing posterior portions of digestive and reproductive systems, and caudal adhering apparatus. $\times 28$.

Fig. 6.—Posterior end of δ *H. siredonis*. Note longitudinal rows of quadrangular tubercles, the crescent-shaped spicules, the spearhead-like gubernaculum, and ten pairs of post anal papillae.

Fig. 7.—Female reproductive system of *Hedruris armata*. Note arrangement of eggs or ovaries and form and position of seminal vesicles. After Perrier.

Figs. 8 and 9.—Eggs of *H. siredonis*. $\times 260$.

ABBREVIATIONS USED ON PLATE

<i>a</i> — Anus	<i>ov</i> — Ovary
<i>au</i> — Ascending uterus	<i>ovd</i> — Oviduct
<i>bu</i> — Branches of uterus	<i>ph</i> — Posterior hook
<i>dl</i> — Dorsal lip	<i>ps</i> — Posterior sucker
<i>du</i> — Descending uterus	<i>psv</i> — Peduncle of seminal vesicle
<i>ep</i> — Excretory pore	<i>sv</i> — Seminal vesicle
<i>int</i> — Intestine	<i>u</i> — Uterus
<i>ll</i> — Lateral lip	<i>up</i> — Uterus proper
<i>lo</i> — Lumen of oesophagus	<i>va</i> — Vagina
<i>nr</i> — Nerve ring	<i>vu</i> — Vulva
<i>oes</i> — Oesophagus	<i>wo</i> — Wall of oesophagus
<i>on</i> — Oesophageal nerve	

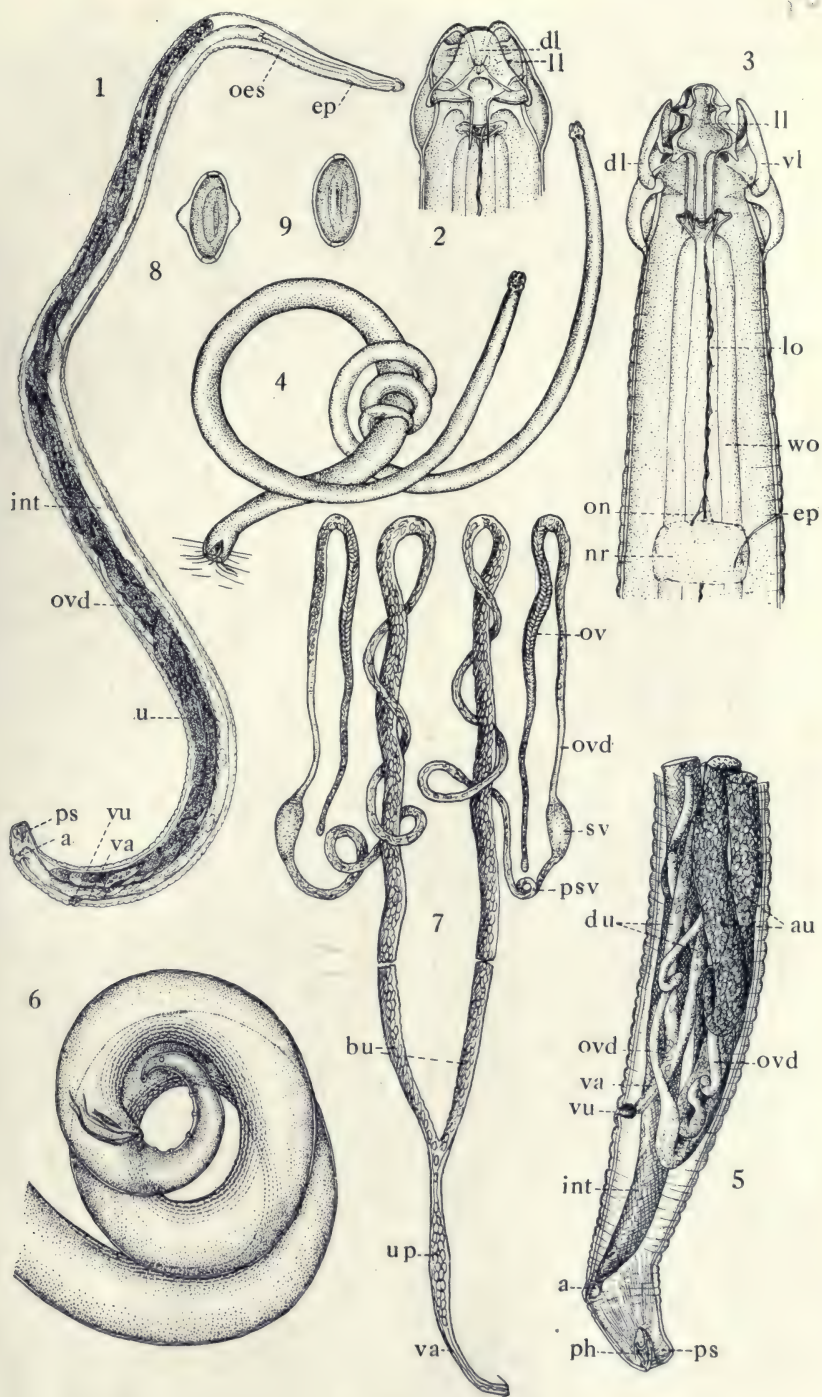
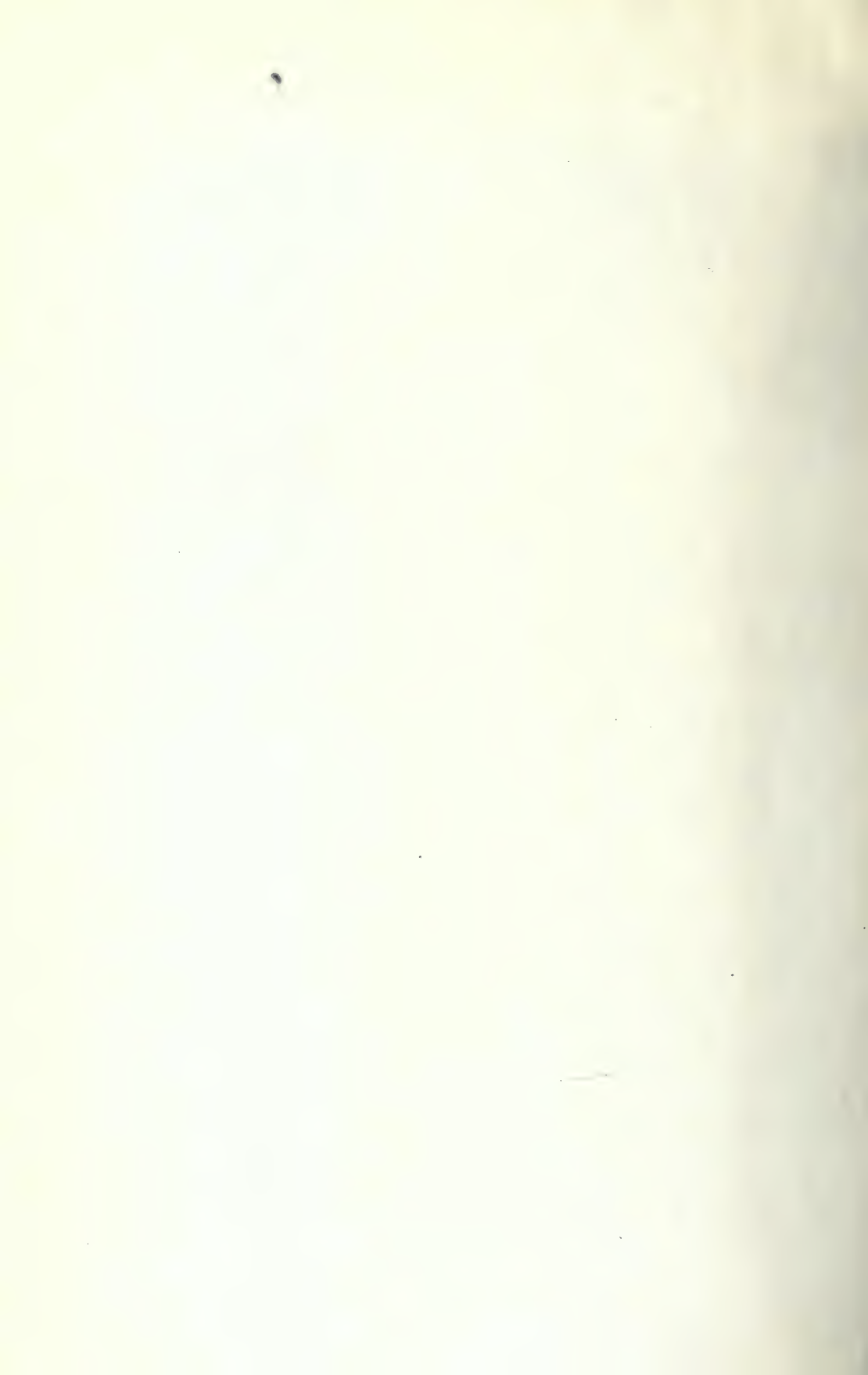


PLATE IX



OBSERVATIONS ON *MICROPHALLUS OVATUS* SP. NOV.
FROM THE CRAYFISH AND BLACK BASS OF
LAKE CHAUTAUQUA, N. Y.*

H. L. OSBORN

The recent account by Yoshida (1916:76-82) of an unnamed trematode infesting the liver, ovary and other tissues of Japanese crustacean, living in burrows between tides on the seashore near Osaka, recalls some observations which I made several years ago, but which have never as yet been published. Though Yoshida did not designate the form which he described, his account and drawings show it to be a member of the genus *Microphallus* (Ward, 1901:184). For convenience, I will refer to it as *M. japonicus*, which name I would propose to call it.

Especial interest attaches to the case of *M. japonicus* as another instance of that remarkable correspondence between parasite and host organ infected which has been noticed by various writers and in particular by Johnston (1913:272) in Australian frogs. *M. japonicus* is the third case now known where the liver or adjoining thoracic viscera of the crayfish or some other crustacean is infected by a species of *Microphallus*.

I first noticed the Chautauqua species in the crayfish in the summer of 1898 and saw its close relationship with *M. opacus* of Ward (1894:173 and 1901:175). I found it later also in the stomach of the black bass and of the bullhead from Lake Chautauqua. In the liver of the crayfish (Fig. 1) it forms large whitish spherical cysts among the tubular cecae of the infected organ. In August, 1902, it was found in every individual of a group of twenty-five examined. In one instance 22 cysts were found on one side and 18 on the other, totalling 40 cysts for the organ. One case, very badly infected, showed about 100 cysts. In the stomach of the black bass partly digested crayfish are often found, and specimens of the worm in various stages are seen which are thus traced to the crayfish as the source of infection. The bass is more often infected than the bullhead, the diet of the latter regularly being more largely molluscan. The Chautauquan form shows marked resemblances to *M. opacus*, but there are certain differences which seem at present to justify distinguishing them and I am proposing to give it the name *M. ovatus* in allusion to its shape.

* Contributions from the Biological Laboratory of Hamline University, St. Paul, Minn.

M. ovatus differs from *M. opacus* in its habits, the latter infecting not the liver but the genital glands and the space above the cephalothorax. The Japanese species infects the liver like *M. ovatus*, and also the ovary like *M. opacus*. The final host of *M. opacus* also is different. Ward examined *Micropterus dolomieu*, "yet none were infected"; though it was found in the yellow perch, but the chief host is *Amia calva*, where hundreds were found in the intestine (vs. stomach in *M. ovatus*) just above the spiral valve.

The cysts of *M. ovatus* as removed from the liver are slightly elongate, measuring 1 mm. by 0.7, slightly smaller than Ward's figures, 1.28 mm. by 0.9, and larger than *M. japonicus*, which averages 0.521 mm. by 0.418. The encysted worm is bent double ventrally; under slight compression enough of its structure is visible to show that it is nearly or completely developed, as shown in Figure 2, drawn from a specimen immediately after its escape from the cyst. Inside the cyst the anterior end of the body can be seen actively moving. The crustacean host is thus evidently an intermediate host interpolated between the primary host and the definitive one, as happens in the case of *Clinostomum* and various other genera of trematodes.

Specimens of the free mature worm from the bass (Fig. 3) had the body densely packed posteriorly with the genital glands, but clear anteriorly so that this part was very mobile, pushing itself forward and seeming to adhere slightly by means of the small oral sucker, while a wave of contraction swept backward the whole length of the body giving it a dumb-bell shape as it passed the center and passed off over the hind end without influencing its form. *M. ovatus* is larger than *M. opacus* and presumably than *M. japonicus*, the adult of which is not known. The dimensions of the body in a mature specimen from the bass were 2.6 by 1.7 mm. Another worm drawn under slight compression measured 3.7 by 1.2 mm. One from the bullhead gave 3 by 1.8 mm. Ward gives 1.7 by 1 mm. for *M. opacus*, and *M. japonicus* from the crustacean cysts in maximum specimens was 0.833 by 0.325 mm. As the organization of the encysted specimens of *M. japonicus* is almost completely developed, one must conclude that the mature worms are much smaller than the American forms. The cuticula is spinous throughout as in the Japanese form, but unlike the *M. opacus* according to Ward, who says (1894) that the entire surface is free from spines.

The terminal oral sucker measured 0.125 mm. in a section of the worm, in another case drawn from a living specimen it measured 0.1 mm. Ward gives 0.155 in *M. opacus*, and Yoshida 0.05 mm. In the size of the ventral sucker, *M. ovatus* also differs from the others,

measuring 0.16 and 0.175 mm. in two cases, as against 0.210 in *M. opacus* and 0.035 in *M. japonicus*. The suckers are relatively larger in *M. opacus* as the bodies are smaller. In *M. ovatus* the ventral sucker is located on the level of the fifth eighth of the body length. It is located at about the same point in *M. opacus*, but in *M. japonicus* it is more posterior, being placed at the sixth eighth of the total length. The genital opening is located on the level of the center of the ventral sucker and in immediate contact with its left side. The excretory opening is placed at the extreme posterior end of the body.

The digestive apparatus is very poorly developed, the least of any of the members of this family. The very feebly developed pharynx, slightly more remote from the oral sucker than is that of *M. opacus*, has a length of less than 0.1 mm. and a diameter of only 0.05 mm. A long, slender esophagus leads back to a small triangular sack which can hardly be said to be forked, thus contrasting with the distinctly developed ceca of *M. opacus* and the still more elongate ones of *M. japonicus*. Sections and total preparations show a feebly developed epithelium in the interior of this digestive apparatus, but no conspicuous glands connected with esophagus.

The dorsally located excretory bladder is V-shaped, each side is broad and conspicuous, measuring about 0.4 mm. in length and having a diameter of about 0.1 mm. In specimens from the crayfish the cavities of these vesicles are filled with highly refractive globules doubtless the stored excretions resulting from the metabolisms of the encysted animal, similar to those found in various other cases of encysted stages (e. g. Faust, 1917: 42), but not found in the mature worms from fishes.

Possibly the drawings of Yoshida (Fig. 2) may be interpreted to mean the same thing. The excretory vesicles of living specimens were watched on several different occasions, but no pulsations or other movements were detected. A slender tube was traced forward from each horn of the bladder to a point on the level of the pharynx. These lateral vessels gave rise to smaller vessels which in turn gave rise to capillaries and terminated in flame cells. As in *M. opacus* and *M. japonicus* the cerebral ganglion and the lateral nerve cords are very noticeable even in preserved preparations.

All of the reproductive organs are confined to the posterior half of the body. The compact globular testes lie nearly opposite each other, they are distinct and not in contact with the excretory vesicles, whereas in *M. japonicus* they are apparently in contact. Their ducts join and form a common duct which, passing anteriorly to the ventral sucker, enters the posterior side of the large seminal vesicle which

lies directly in front of the ventral sucker. The organ is also conspicuous in *M. japonicus*, where it is called by Yoshida "the semilunar organ." In mature specimens it was filled with living spermatozoa. It tapers distally (see Fig. 5) to form a passage leading directly to the eversible penis and surrounded by cells of the prostate glands. As in all the Microphallinae, these parts are not enclosed in a cirrus sack after the manner of many trematodes. There is no atrial pocket common to the penis and metraterm as in *Levinseniella* (Lühe, 1899: 124), but these organs reach the surface entirely separately, as shown in Figure 4. The globular ovary, larger than the testes, lies on the level of the ventral sucker and on the right side of the body. Its duct meets the vitelline ducts close behind the ventral sucker as in *M. opacus*, there is no seminal receptacle, the uterus in mature stages is much enlarged and filled with eggs, in encysted stages from the crayfish its empty windings can be seen. The course of the uterus in a mature specimen is shown in Figure 3. After leaving the yolk receptacle it runs to the posterior end of the body, then bending, runs forward and back again on that side externally to and partly enclosing the vitellaria it then crosses to the opposite side and again runs forward and back in close relation with the vitellarium; finally it runs directly forward to the genital opening. The uterus contains great numbers of small dark-shelled operculated ova which measure 25 by 12 μ , slightly smaller than those of *M. opacus*, which, according to Ward, measure 0.03 to 0.04 by 0.015 to 0.02 mm.

The vitellaria are more compact apparently than in *M. japonicus*, where the follicles are shown as if quite distinct and apparently are less deeply lobed than in *M. opacus*. They are located externally to the spermaries and extend beyond them reaching as far anterior as the level of the ventral sucker. Their position is intermediate between that of the *M. opacus* and *M. japonicus*, where they are wholly behind the spermaries or wholly anterior to them, respectively.

The habits of *M. ovatus* are slightly different from those of *M. opacus*. From the accounts of Ward one would consider that the liver of the crayfish is not infected, as the infection is said to be seated in the space above the cephalothorax and sexual organs. The final host also is different as shown by Ward who examined *Micropterus dolomieu*, "yet none were infected," though it was found in the yellow perch, but it is found in *Amia calva* where hundreds were found in the intestine just above the spiral valve. We note also that the Japanese species inhabit the liver of its crustacean host like *M. ovatus* as well as the "ovary and hypodermis" like *M. opaca*.

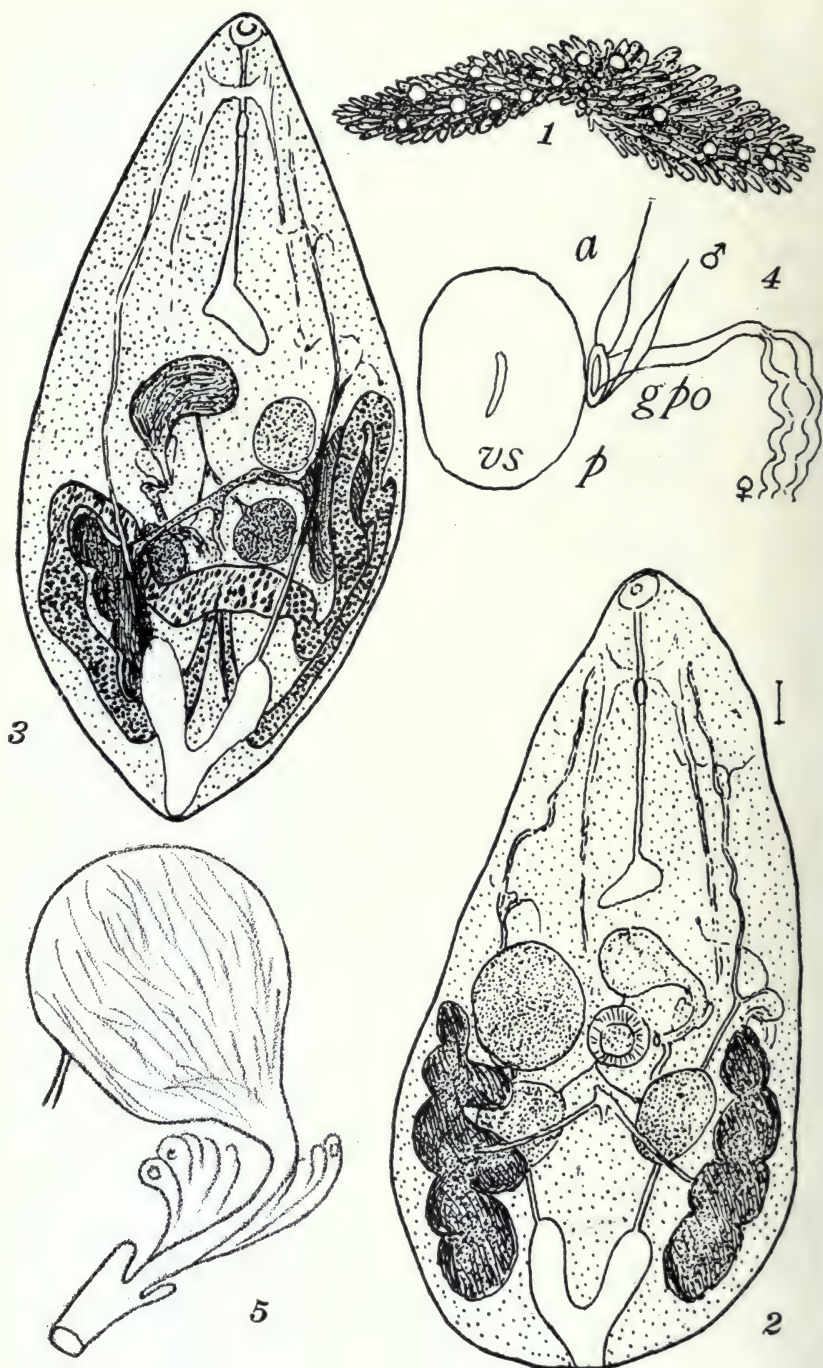


PLATE X

The differences noted between these three species are shown briefly in the following table.

<i>Microphallus</i>	<i>Opacus</i> Ward	<i>Japonicus</i> Yoshida	<i>Ovatus</i> Osborn
Infect as larva	Genital glands, cephalothorax of <i>Cambarus</i>	Liver, ovary and cephalothorax of <i>Helice</i>	Liver, of <i>Cambarus</i>
Adult	Intestine of <i>Amia</i>	Stomach of bass
Maximum length	1.7 mm.	0.85 mm.	3.7 mm.
Cuticula	Non-spinous	Spinous	Spinous
Ventral sucker	0.210 mm.	0.175
Intestinal ceca	Distinctly formed	Moderately long	Very rudimentary
Vitellaria	Deeply lobed, wholly posterior to testes	Wholly divided; wholly anterior	Slightly lobed, enveloping testes

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EXPLANATION OF PLATE

Fig. 1.—View of the liver of *Cambarus* from Lake Chautauqua, N. Y., showing the cysts of *M. ovatus* in place among the tubules of the organ.

Fig. 2.—Ventral view of immature specimen of *M. ovatus* immediately after its escape from the cyst, based on camera lucida drawings from compressed living specimens and total preparations. Scale equals 0.1 mm.

Fig. 3.—Dorsal view of mature specimen, taken from the stomach of *Micropterus dolomieu*, showing the location of the coils of the uterus well filled with embryos, from free hand drawings.

Fig. 4.—View showing the relation of the ventral sucker, genital opening, penis and metraterm; drawn from living compressed specimen.

Fig. 5.—View from living specimen showing form of the seminal vesicle, prostate glands and eversible penis.

A NEW CYSTOCERCOUS CERCARIA *

H. S. PRATT

The cercaria described in this paper was found in the liver of *Goniobasis livescens* taken in the Oneida River near the outlet of Lake Oneida, in the State of New York. To it may be given the name *Cercaria fusca*. Twenty-three snails of the above mentioned species were examined, and of these, four were infected with small numbers of this cercaria. The color of the living worms is brown, the tail being much darker brown than the body. They were very sluggish when taken from the snails and moved very little, and made no swimming motions. The length of the extended live cercaria was about 3 mm., the body being about 1 mm. in length and the tail 2 mm.; the length of the flat tail-forks was about 0.5 mm. (Fig. 1a). Sporocysts were also found in the snails, which contained each from one to four or five cercaria in various stages of growth. The sporocysts are mostly lenticular in shape and 2 or 3 mm. in length. The largest of them contained but a single cercaria, which thus had the appearance of being encysted. One sporocyst observed, in which were several cercariae of different sizes, contained one apparently full grown, the tail of which projected freely from the sporocyst, giving the impression of a sporocyst with a tail.

These observations seem to support the statement of Faust (1918: 149) that the cystocercous cercariae may be cannibalistic. The largest cercaria in a sporocyst seems to feed upon, or at least absorb, the smaller ones until it is finally the only one left. I did not observe in the material studied any instance of the direct attack of a large cercaria upon a smaller one.

Cercaria fusca is similar to *C. brookoveri* Faust, 1918, in that the young distome is not surrounded by the walls of the anterior portion of the tail, as is the case in other cystocercous cercariae observed, but is joined with the tail by a short fold.

The shape of the body of the full-grown cercaria is pyriform (Fig. 2), the hinder end being the broader, where the width is 0.65 mm. The cross section is oval in shape, the thickness being 0.40 mm. The suckers are large. The oral sucker is an elongated organ 0.52 mm. long, 0.35 mm. wide and ventral in position. The acetabulum is

* This study has been made as a part of the ecological survey of Oneida Lake, being made under the direction of Prof. C. C. Adams of the New York State College of Forestry, Syracuse, N. Y.

just behind the middle of the body and measures 0.24 mm. in diameter. The pharynx has a diameter of 0.13 mm. and opens into a very short esophagus, which in turn leads into the two limbs of the intestine, each of which passes in the lateral area of the body to its hinder end. They are filled with a translucent secretion.

The median excretory trunk is short, passing from the hinder end of the body to a point immediately back of the ovary and dorsal to the testes, from which the two lateral limbs pass forwards in the dorsal area of the body to the right and left of the acetabulum. The testes and ovary are all spherical organs forming a group immediately back of the acetabulum and near the hinder end of the body. The two testes are close together in the same transverse plane, and in the ventral area of the body; each has a diameter of 0.13 mm. A large cirrus sac lies dorsal to the acetabulum. The ovary is slightly smaller than either of the testes and lies between and in front of them towards the dorsal

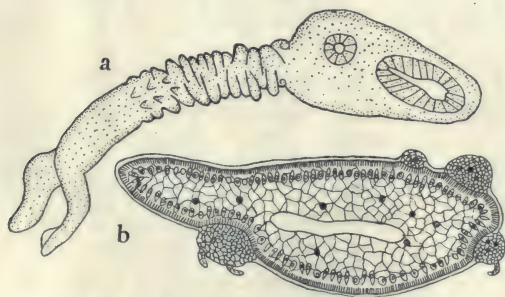


Fig. 1.—(a) *Cercaria fusca*, $\times 42$. (b) Cross section of anterior portion of tail, $\times 400$.

side of the body. A large receptaculum seminis lies alongside of the ovary. The uterus passes forwards to the oral sucker and thence back to the genital pore, immediately in front of the acetabulum. It contains a few large eggs which average about 78 by 49μ in size. The full grown cercariae are thus sexually mature and in this respect resemble *C. macrostoma* Faust.

The vitelline glands occupy the two lateral areas of the body from the oral sucker to the hinder end of the body, and consist of a large number of small follicles.

The tail of the full grown cercaria is flattened in shape and has a width of 0.27 mm. and a length of 2 mm. Each of the two forks of the tail is 0.5 mm. long and 0.27 mm. wide at its base. The tail is made up of two very distinct regions, the anterior two-thirds and the posterior third. The former region is distinguished by the presence of conspicuous warts and transverse ridges on its surface, similar to

the warts which have been observed in other cystocercous cercariae. It is also considerably thicker than the posterior region, having an oval cross section (Fig. 1, b). In the anterior portion is a large open space in the parenchyma which represents the excretory trunk.

The parenchyma of the tail is large meshed with numerous nuclei, and contains very conspicuous subcuticular cells and longitudinal and circular muscle fibers. The longitudinal fibers are wide bands lying close together and extending the length of the tail; it is by the contraction of them that the animal exercises the energetic swimming motions observed by Ward in *C. anchoroides* (1916:12). The subcuticular cells form a very regular row which lies just within the longitudinal muscle fibers. They are mostly elongate or pear-shaped

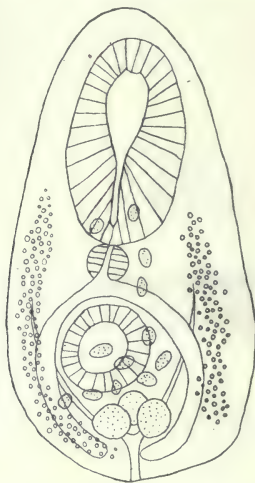


Fig. 2. Ventral aspect of distome with internal organs, $\times 125$.

cells, the pointed ends of which extend towards or between the longitudinal muscle fibers. The warts contain no subcuticular cells and no muscle fibers but are composed entirely of a very close-meshed parenchyma in which lie a few nuclei, bounded on the outer surface by a cuticula. On the summit of many of the larger warts are one or two short, curved, finger-like projections. The circular and longitudinal muscle fibers and the subcuticular cells pass across the base of the warts, which thus lie entirely outside of them.

The posterior third of the tail together with the two flat tail-forks are similar in structure to the anterior two-thirds, except that they are much flatter and lack the warts. They also lack the wide excretory space of the anterior portion. Instead of this a narrow excretory canal passes through the middle, and branching at their base extends to the end of each of the forks.

Cercaria fusca is similar in structure to *C. macrostoma* Faust, and like that worm probably belongs to a species of distome allied to *Allocreadium*. It is also probable that *C. macrostoma* escaped from *Goniobasis pulchella*, inasmuch as that snail was present in the aquarium in which the worm was found swimming (Faust, 1918: 150).

The very regular arrangement of the subcuticular cells in the tail of *C. fusca*, as above described, and their frequent elongation between the longitudinal muscle fibers towards the cuticula would seem to give weight to the theory, so popular at present among helminthologists and morphologists generally (see Pratt, 1909), which regards them as a modified hypodermis, the function of which is to secrete the cuticula. The structure of the warts, however, does not give support to this theory, but disproves it, in that they contain no subcuticular cells but yet possess a well-defined cuticula. The subcuticular cells which pass along the base of a wart are separated from it by both the longitudinal and circular muscle fibers and bear no relation to it. They are portions of the parenchyma of central portion of the tail.

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OBSERVATIONS UPON *TRICHOMONAS INTESTINALIS* IN VITRO

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In a recent communication to this Journal I reported a successful cultivation of a strain of *Trichomonas intestinalis*. It is the purpose of the present communication to record some further observations upon this intestinal flagellate.

Fecal suspensions for subculturing the strain have been continuously secured from the same individual, but I have found that certain specimens of feces are unsuitable for the purpose of making suspensions, since in these the organisms will not grow. These unsatisfactory suspensions are characterized by an acid reaction to litmus and an intense bile staining of the saline above the fecal sediment. On the other hand, the suspensions in which good growth has been secured have been neutral to litmus and the supernatant saline has been colorless or only tinged slightly yellow.

I now prepare the fecal suspension as follows:

A soft portion of the fecal mass is selected and thoroughly triturated in about ten volumes of saline and strained through cheese cloth to remove the coarser particles of fecal debris. The suspension is then centrifuged until the supernatant fluid is clear. If not bile stained and if its reaction is neutral, about one cubic centimeter of the sediment is added to each of a number of test tubes containing about ten cubic centimeters of physiological saline. Without sterilization these are employed for subculturing, one or more being reserved as controls to indicate that flagellates are not present in the feces employed in making the suspension. If the supernatant wash fluid were bile stained it might be possible to secure a satisfactory suspension by one or more washings to remove the excess of bile, but so far I have not had to do this. The suspension thus consists of living fecal bacteria and small particles of undigested or partially digested food. The bacteria prey on the latter and the trichomonads prey on the former. In old cultures there is a very noticeable reduction in both the bacterial and food debris.

Since its cultivation *in vitro* our strain of *Trichomonas* has been carried through subcultures as follows:

No.	Source	Age of Parent Subculture at Time, Days	Greatest Age at which Motile Flagellates Found, Days	Greatest Age at which Sub- cultures Gave Growth, Days
1	Original	..	59	60
2	1	13	46	47
3	2	18	28	65
4	Failure *
5	3	29	27	..
6	5	24	4	..
6	3	65	9	..
7	6	9

* Probably due to excess of bile in feces.

From the foregoing it may be seen that these cultures retain their vitality, as measured by the presence of active flagellates, for a period which compares favorably with those of some of the more rugged pathogenic bacteria when growing *in vitro*. Not much growth is evident within the forty-eight hours following a transfer, but it apparently reaches a maximum when the culture is from four to five days old. A loopful of the sediment, placed on a slide and flattened out with a cover glass, may with the low power, reveal nearly a hundred flagellates per field. Following this, their numbers undergo a steady diminution, until not over two or three will be observed in an entire preparation made from a loopful of sediment. I have reason to believe that this diminution in the numbers of flagellate forms is due to encystment.

My observations have convinced me that this organism is *Trichomonas intestinalis*, though as noted before, I have failed to observe an axostyle in the fixed preparations I have made. Nevertheless, an axostyle is distinctly visible in the living organism, projecting posteriorly as a rigid spinous process, as shown in the outline drawings in the plate herewith. The axostyle apparently serves as an organ for temporary attachment. I have observed individuals attached by its assistance to the cover glass or food debris twist and rotate upon their axostyle very much in the manner of a vorticella. When fully active the three anterior flagella move at such a rapid rate they cannot be seen. However, in sluggish individuals they are distinctly visible, and it can be seen that their movement partakes of a rotary character, as indicated in Figure 4. The anterior portion of the undulating membrane is rarely visible during life, but the posterior portion, apparently having a longer membrane, can readily be seen. In very sluggish individuals its movements are suggestive of the movements of pseudopodia, especially where the flagellate is in the pre-encysting stage. From observations of living organisms, it seems probable that the idea these organisms may project pseudopodia, as suggested by Wenyon (1915) may have arisen from observation of the movements

of the posterior portion of the undulating membrane in sluggish individuals. In the pre-encysting state, when the organism, deprived of all appendages, seems to possess a distinct ectoplasmic-endoplasmic differentiation and thus somewhat resembles a small amoeba, the sluggish movements of the undulating membrane are still more suggestive of pseudopodia. However, the ectoplasmic bulgings, representing the altered undulating membrane, first appear toward the anterior extremity and slowly progress posteriorly. These are indicated at the arrows in Figures 7, 8 and 9.

In stained preparations the organism is seen to possess three anterior flagella, and a single posterior flagellum, having an origin at the same place as the others, but projecting posteriorly and forming the free edge of the undulating membrane. It may extend beyond the posterior extremity of the cell body as a free flagellum. A distinct chromatic line at the base of the undulating membrane is visible. All four flagella arise from an anterior blepharoplast or kintocynucleus. The cytostome has not been observed in any of the individuals examined, neither have chromatic blocks been observed about the nucleus. The interior of the cell body invariably contains food vacuoles of varying size, in many of which can be observed bacteria in various stages of digestion. As previously noted, I have not observed the axostyle in fixed specimens, though it is clearly visible in the living animal.

Multiplication appears to be by longitudinal division, an example of which is indicated in Figure 5, in which there can be observed two nuclei, two sets of flagella and two chromatic lines, but cleavage of the cell body has not yet occurred.

In an endeavor to see if the culture strain could re-establish itself in an animal, two white rats were selected, and by fecal examination determined to be free from trichomonads. One rat (A) was given one feeding of milk to which had been added a portion of Culture 2, at that time forty-two days old. Not over one motile flagellate was present per slide preparation, while the culture chiefly contained the spherical bodies later recognized as cysts. The other rat (B) was kept in another cage as a control, receiving similar feed and treatment, but was not fed infected food. Six days later a single motile trichomonad was observed in preparations made from a fecal pellet of Rat A, while similar preparations from Rat B were negative. The entire remaining fecal pellets from both rats were transferred to tubes of saline and separately suspended therein. Three days later these cultures were examined, and from four to five trichomonads were observed per field in the culture from the feces of Rat A, while none were present in the culture from Rat B. Fourteen days after the infected feeding, both

rats were killed. The results of the examination of Rat A (infected) were as follows: No flagellates were observed in the feces, but spherical bodies regarded as cysts were present; the cecum was full of soft pultaceous feces, which was literally swarming with *myriads* of trichomonads; no trichomonads were observed in the small intestine or stomach. Examination of Rat B (control) was entirely negative for trichomonads. The cecal contents of both rats were transferred to saline. When examined three days later, numerous trichomonads were present in the culture from Rat A, but in numbers much less than in the fresh cecal contents. The culture from Rat B was negative. Motile flagellates were still present in the culture from A when examined a month later.

The mucosa lining the cecum of Rat A appeared perfectly normal and throughout the course of the experiment the rat appeared to enjoy unimpaired health.

In studying the growth in cultures I had at first difficulty in recognizing the trichomonas cysts. Various observers have described objects they regarded as trichomonas cysts, a number of which are figured by Lynch, but the chief claim in favor of each appears to be the fact they have been observed in material in which trichomonads were present, and hence were assumed by the observers to be cysts of the observed protozoan. These conflicting views were found confusing. I finally found that by staining the living trichomonads with dilute neutral red solutions and allowing the preparations containing them to undergo gradual desiccation, cyst formation invariably took place and all stages the process could be observed. Figures 7 to 14 represent the process of cyst formation from the flagellate stage in one individual, while Figures 15, 16 and 17 represent typical cysts. The cysts are perfectly spherical and from 5 to 6 micra in diameter. In some a peripherally located nucleus can be discerned. In the drawings the relative thickness of the double contoured wall is somewhat exaggerated. In cultures, without the aid of neutral red solutions to stain the food vacuoles, the cysts can only be made out with difficulty, since their walls are not highly refractile. I am not satisfied I have as yet observed them in fixed films.

It remains to be ascertained whether infection by feeding results from the flagellates or from the cysts. By analogy the latter would seem probable and my single feeding experiment, taking into consideration the paucity of flagellates present, would tend to support this view.

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EXPLANATION OF PLATE

All figures are free hand drawings

Figs. 1, 2, 3, 4. Unstained living trichomonads. 1, 2, and 3 show various changes of position of a trichomonad which has temporarily attached itself to the coverslip by the tip of its axostyle. Movements consist of a rotation on its long axis as well as lateral flexion. Flagellar movement is too active for visibility. 4, outline of a trichomonad whose movements were sluggish. The three anterior flagella rotate as indicated by the arrows. At (a) the movements of the undulating membrane are most distinct. When movement of the membrane temporarily ceases, the change in the organism's shape is indicated by the dotted line.

Figs. 5, 6. From smears fixed in sublimate alcohol and stained with Heidenhain's iron hematoxylin. 5, beginning longitudinal division. Cleavage of the cell body has as yet not taken place. 6, a typical trichomonad.

Figs. 7 to 17. Cyst formation. Living trichomonads from culture stained with dilute neutral red. The food vacuoles stain a dark cherry red and the nucleus appears as a pale pink disk. Figs. 7-14, progressive changes in cyst formation. Changes observed in a single individual. 7. Pre-encysting form. Sluggish, no flagella observable, movements of undulating membrane, indicated by arrow, very sluggish and resemble pseudopodia. 12:20 p. m. 8. Some contraction of the body. Axostyle retracted. Undulating membrane still moving. 12:50 p. m. 9. Distinct differentiation into ecto- and endo-plasm. Undulating membrane moving slightly. 1:15 p. m. 10. Some extension of ectoplasm, approaching original shape. 1:40 p. m. 11. Ectoplasm again contracting. 1:45 p. m. 12. Nucleus no longer visible. Outline nearly spherical. 1:50 p. m. 13. Peripheral ectoplasm becomes denser, outline distinctly double contoured. 1:52 p. m. 14. Perfectly spherical, double contoured wall. Clear zone of ectoplasm has disappeared. Cyst complete. 1:57 p. m.

Figs. 15, 16, 17. Typical cysts. Nucleus in peripheral situation is visible in 15. Food vacuoles visible in all. Diameter from 5 to 6 micra.

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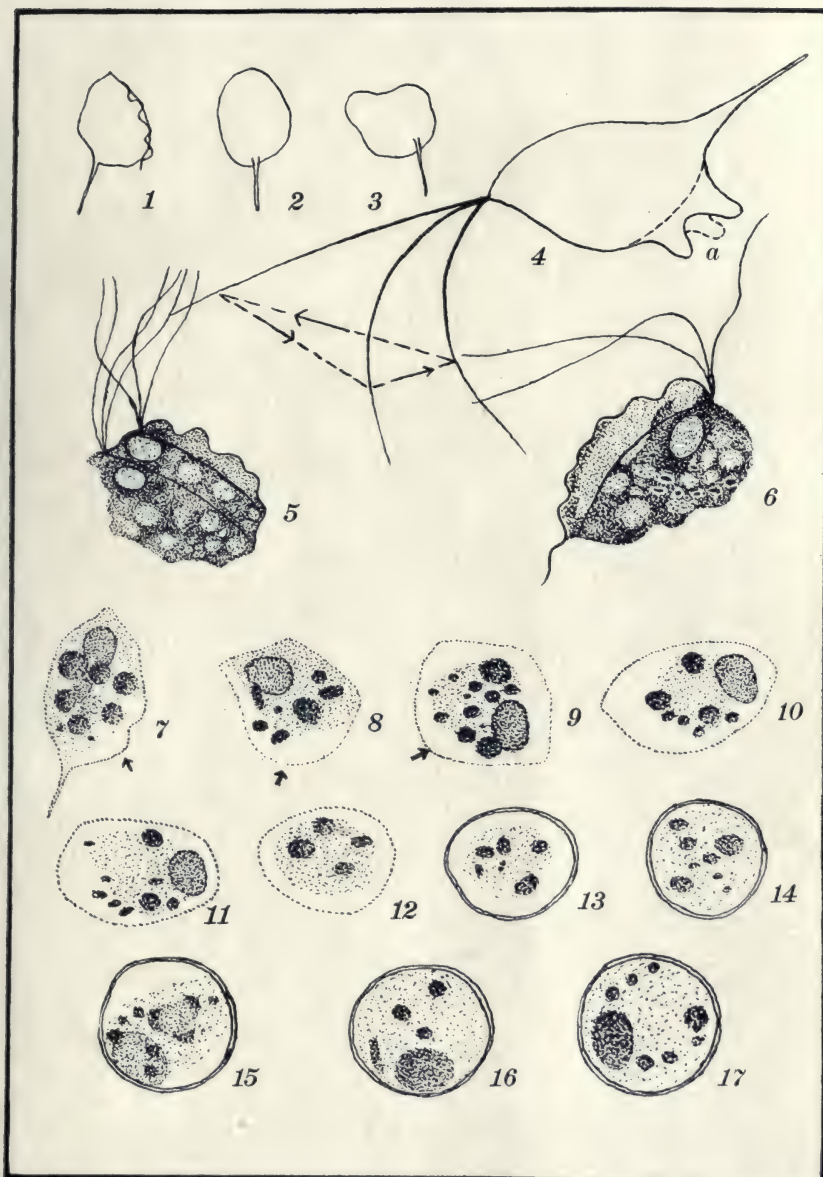
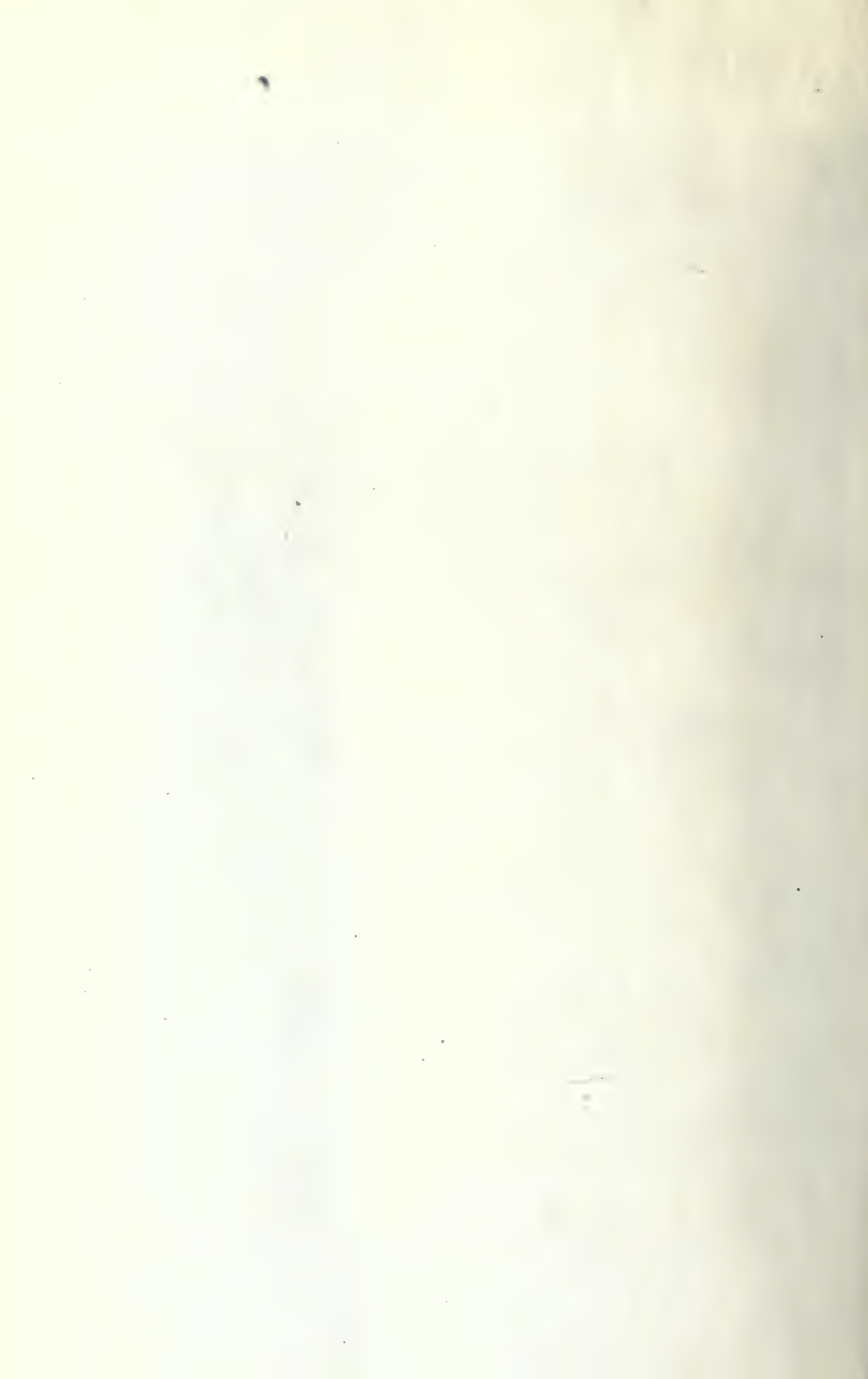


PLATE XI



A CASE OF *BALANTIDIUM COLI* DYSENTERY

C. W. MASON, M.D.

CHIENG RUNG, YUNNAN, CHINA

Human balantidiasis has been considered extremely rare, but I believe is less so than is usually supposed. Strong (1905) was able to find only 125 reported cases. Walker (1913) claims that "In the Philippine Islands, however, parasitizations with this protozoa appears to be relatively prevalent. The first case here (P. I.) was by Strong in 1904. Subsequently a few cases were reported, notably three fatal cases with necropsy by Bowman (1909 and 1911). Willets (1913) found two cases in examination of 400 stools, and I (Walker) found two cases in the examination of 48 stools. Thirteen cases have been observed in the Philippine General Hospital. In the Bilibid Prison, thirty-five cases have been found in the last two and a half years, an average of more than one a month. From March 4 to March 25 of the present year (1913), eight new cases of parasitization with the protozoan were discovered. However, on account of the infrequent appearance of the parasites in the stools of infected persons and the absence of clinical symptoms in many of the cases, it is probable that parasitization with *Balantidium coli* is frequently overlooked in the routine examination of stools."

For a classical and full description of the etiology and pathology I know of nothing equal to this article of Walker. And anyone interested should obtain that article; but I feel that some of his conclusions are important enough to be quoted here.

"*Balantidium coli* was never found entering the tissues through the lesions in 10 parasitized monkeys having a colitis or ulcerations due to bacteria or other causes.

"In those monkeys in which infection took place, the balantidia entered the tissues through the sound intestinal epithelium.

"*Balantidium coli* produces bacteriologically sterile abscesses in the submucosa of an infected intestine.

"*Balantidium coli* is the primary etiologic factor in the symptoms and lesions of balantidial dysentery.

"The latency prevalent in balantidiasis of man is due chiefly to the fact that the patient, although parasitized, is not infected with *Balantidium coli*, but in part to the chronicity of the ulcerative process in infected cases.

"Every person parasitized with *Balantidium coli* is liable sooner or later to develop balantidial dysentery."

In my routine examination of stools I have seen several infections with this parasite, but I was misled by statements in some of the older books that parasitization with this parasite was of no clinical importance, so I made no record of the number of infections noticed. And so far I have only seen this one case in which there was any serious symptoms.

CASE. E. B., male, aged 30, a Danish missionary in Southwestern Yunnan Province, China. He had been living in most unhygienic surroundings, having a Chinese family, including all their pigs, etc., occupying a part of the same house. Flies in enormous numbers swarming on his food at meal time. He had brought his wife to me for confinement. He was a strong, well nourished individual and boasted that he had never taken a dose of medicine. While here he came down with an attack of malaria fever. He was convalescing from this when he began having dysenteric stools. In appearance and odor these seemed to be a typical case, and as amebic dysentery is endemic here I naturally suspected that. He had from twelve to fifteen movements in twenty-four hours. He did not tell me until the third day of his disease, he seemed to be ashamed that he was ill. I then made a microscopical examination of the stools and found them swarming with *Balantidium coli*. I could find no ameba either active or encysted and the specimen seemed almost free of bacteria.

Treatment.—Being at a loss as to a specific against this infection, my mind naturally turned to the weapons used to fight amebic dysentery. At the suggestion of Dr. M. E. Barnes of the International Health Board (Rockefeller Foundation), Chiangmai, I had been trying the oil of chenopodium as an amebicide in amebic dysentery. His suggestion was to give the oil in solution with castor oil by mouth. I had modified it by dissolving it in olive oil and giving 50 to 60 minims in a half ounce of olive oil injected just inside the internal sphincter and I had found that reverse peristalsis soon relieved the desire to go to stool. It seemed logical that as this disease is entirely a disease of the lower bowel the results should be quicker and more efficacious as the drug used would not be subjected to the chemical laboratory actions of the entire alimentary tract and would be in higher concentration. So I gave him an enema of 60 minims oil of chenopodium in half an ounce olive oil. He retained it two hours. The very first movement had fecal matter in it. The second was a formed motion. Next day he was apparently well. Had only two motions both formed and no blood or mucus to the naked eye. The third day I found a few very sluggish *Balantidia* in his stool and repeated the same enema. Six days later he had a slight relapse but reported it at once and I gave him a third dose. He left me three weeks later with no symptoms and the stools were negative on several examinations. I feel that there is a grave chance of a return of his trouble, but I wish to bring before my colleagues this fact, that he made such a rapid and complete symptomatic recovery and that without any particular restriction of diet except to withhold fruits and coarse rye graham bread for a few days. I fully realize that this was a purely empirical treatment and I pass it on that others may do the scientific experimenting with this drug. The main thing to me or any practitioner was, my patient got well.

PAPERS CITED

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Walker, E. L. 1913.—Experimental Balantidiasis. Phil. Jour. Sci., 8B: 333-350.

NEW HUMAN PARASITES

THE JOURNAL will endeavor to print very promptly brief reference to all new species of human parasites published since January, 1919. The cooperation of authors and investigators will materially assist in making this of value.

Oxyuris incognita Kofoid and White, 1919. This species of nematode is based on ova found in feces of 1.2 per cent. of about 30,000 soldiers at Camp Travis, Texas, infected individuals coming from 22 states. Adult worms were not discovered. (Jour. Am. Med. Assn., 72:567, Feb. 22, 1919.)

Leptospira sp. Noguchi, 1919. This protozoan organism, closely resembling that previously found in cases of infectious jaundice, was isolated by Noguchi from guinea pigs inoculated with the blood of yellow fever patients (in 6 out of 27 cases studied at Guayaquil, Ecuador) and apparently the same organism was found in the blood and liver of human patients. It was also found in guinea-pigs inoculated with blood of experimentally infected animals after its passage through a Berkefeld filter. The guinea-pigs showed symptoms and lesions suggestive of those of yellow fever in the human subject. (Jour. Am. Med. Assn., 72:187, Jan. 18, 1919.)

Dicercomonas soudanensis Chalmers and Pekkola, 1919. This species was discovered in human feces in Khartoum (Sudan) in cases of diarrhea, but is not regarded as pathogenic. The article includes a brief but very important discussion of the classification of the Tetramitidae with the modifications involved by the addition of this new form. (Jour. Trop. Med. Hyg., 22:29, Feb. 15, 1919.)

Isospora hominis Rivolta, 1878.

Eimeria wenyoni Dobell, 1919.

Eimeria oxyspora Dobell, 1919.

Eimeria (?) sp.

The coccidia parasitic in man are revised by Dobell; he recognizes four species, two of which are new. *I. hominis* appears to be the most common, about 70 definite cases having been recorded. The cases found since 1915 were in men who had been in Egypt, Gallipoli, Salonika or Mesopotamia. *E. wenyoni* has been found four times in persons from the eastern Mediterranean region. *E. oxyspora* has been found only once in a young man who has been in South Africa, Ceylon and India. These three forms are known only from the cysts found in the feces of affected persons, but their habitat is probably the small intestine. The fourth species discovered by Gubler (1858) is the hepatic coccidium of man very imperfectly known, but apparently belonging in the genus *Eimeria*. Since the first case found in Paris four others have been recorded in Prague, Vienna, Giessen and London, none having been reported since 1890. The hepatic parasite is the only one of the four known to be seriously pathogenic. (Parasitol., 11:147-197; Feb., 1919.)

REVIEW AND NOTES

An extensive and thorough study of the Tsutsugamushi disease has been published in recent numbers of the *Kitasato Archives of Experimental Medicine* by T. Kitashima and M. Miyajima. The concluding section which appeared in December, 1918, includes data on the biological investigations of the virus and a zoological discussion of the mite which acts as intermediate host, and of the field mice which serve as a reservoir of the virus. The authors have reached the positive conclusion that on the basis of the nature of the virus, which is ultra-microscopic, the disease must be classed with the acute infectious diseases among which Spotted Typhus and Rocky Mountain Spotted Fever are to be placed. The number is illustrated by ten splendid plates which include among other things admirable representations of the mite and of the pathology of the disease.

The United States Interdepartmental Social Hygiene Board appropriated from the Scientific Research Fund to Stanford University Medical School, \$7,200, and to the University of Michigan, College of Medicine, \$6,000 for research work on various problems in venereal diseases. It is in position to make further grants under stated regulations to institutions fitted to carry on such research.

Thirty days after the treaty of peace shall have been signed a congress of Red Cross delegates is to be held in Geneva under the auspices of the International Committee of the Red Cross. The campaign it is proposed to inaugurate at once contemplates a world movement for the prevention of disease as well as for its relief. Parasitology is likely to assume an important place in the discussions of the conference.

The current scientific press records the death, on February 7, of Professor Raphael Blanchard of Paris, the distinguished parasitologist, editor of the *Archives de Parasitologie* and noted for a long series of investigations and publications in this field. The Journal will publish in a later issue an article on Professor Blanchard's life and work.

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FASCIOLOPSIS BUSKI

A PARASITE OF MAN AS SEEN IN SHAOHING, CHINA *

F. W. GODDARD

THE DISEASE

Fasciolopsis infestation of man, for many years a medical curiosity, has within the last decade come to be recognized as a serious condition both for the individual and for the community concerned. The first flukes of this type to be described were discovered by Busk in the intestines of a Lascar sailor dying in England in 1843. The parasite has been found in a few other cases in Europe and America, but apparently the disease is endemic only in tropical and subtropical countries. As these comparatively little known regions become more deeply penetrated by the medical missionary and others, infestation of man by this species or related ones is found with increasing frequency, and is now known to occur in India, Assam, Siam, Natal, Borneo, Straits Settlements, Sumatra, Cochin-China, Tonkin, and along the coast of China (Canton, Hongkong, and as far north as the Yang-tse Valley, where in the Shaohing district it is particularly prevalent). In pigs *Fasciolopsis buski* has been reported to be very common in Hongkong and Tonkin. In man, at least in Shaohing, the disease appears to be more common in early life, 5 to 20 years, but it is not rare in persons up to middle life, and we have found it in one infant but a year old.

The prevalence of this disease in any community will in all probability depend on the dietetic habits of the people. Dobson reports that 1 per cent. of the stools of 1,000 coolies examined in India showed the eggs present; Mathis and Léger speak of the "extreme rarity" of the infection in man in Cochin-China. In Shaohing, China, it is extremely common. In the seventeen months from January, 1908, to May, 1909, the diagnosis of fasciolopsis infection was confirmed by microscopic examination of the feces in 51½ per cent. of all the dispensary patients, about 2 per cent. more presented very suggestive symptoms, and doubtless many others were harboring the parasite, though without symptoms. Shortly after the in-patient department of the hospital was

*From the laboratory of Comparative Pathology of Harvard University.

opened nineteen out of twenty patients, or 95 per cent., showed ova in the feces, though none of the twenty was admitted for that condition, nor even suspected it. During the years 1914-16 out of 304 cases admitted to the Christian Hospital, and in which routine fecal examinations were made, 87, or 28 per cent., were found positive for the presence of fasciolopsis.

Three stages may be recognized clinically.

1. *The Period of Latency.*—This period is without notable symptoms and may occupy months or even years. A few flukes in the intestine seem to cause no inconvenience, and how severe the infection needs to be before giving rise to symptoms it would be impossible to say; but in the Christian Hospital the number of flukes recovered from the stools of an individual has varied from a few tens to over three thousand (3,328), and while in general the severity of symptoms is proportional to the number of flukes present, there is also a marked difference in the degree of resistance that an individual may possess, some from whom but a few hundred were recovered being clinically in worse condition than those who had harbored as many thousands. It is probable that asthenia in varying degree, and a mild anemia may appear toward the termination of this stage.

2. *The Period of Diarrhea.*—During the second stage diarrhea appears, and is the condition for which most often relief is sought. There is generally a history of five or six stools a day extending over a period of months, and often with intermissions of days or weeks during which the bowels act normally. The stool itself is usually light yellow in color, without any evidence of blood even under the microscope in uncomplicated cases. It contains a considerable amount of undigested matter, and has a peculiarly offensive odor. Anemia now becomes noticeable and may be extreme, and in Shaohing the combination of anemia with chronic diarrhea is practically pathognomonic of fasciolopsis infection. Other symptoms are inconstant. The appetite may be impaired or even increased, but is usually unaffected; occasionally there may be dull aching pain distributed throughout the abdomen or localized in the duodenal region; temperature, pulse, and respiration do not in uncomplicated cases differ from the normal. In infants and young children the abdomen is very protuberant, and may be the first evidence of disease noted by the parents.

3. *The Period of Edema.*—During the third and final stage anemia is always marked but the most prominent as well as most distressing symptom is edema. This usually affects first the abdominal cavity, then extends to the genitals (which may be very greatly distended), and to the lower extremities beginning in the feet and ankles but soon involving the whole limb, and finally appears in the upper extremities,

the face and the lungs, and with these advanced conditions insufficiency of the cardiac valves may supervene. To relieve the dyspnea due to the ascites it may be necessary to resort frequently to paracentesis abdominis, sometimes as often as every five or six days for a considerable period. The urine is normal except for undue concentration, and even under diuretics may be reduced to but two or three ounces in twenty-four hours. The skin has a yellowish tinge, is harsh and dry, the tongue is glazed, the temperature has a tendency to fall one or two degrees below normal, the patient become extremely weak and death when it occurs is apparently due to exhaustion.

In the treatment of this condition turpentine, oil of eucalyptus with chloroform, thymol and beta-naphthol have all been used with success, the treatment being in fact the same as for hookworm. Personally, I favor beta-naphthol, and believe it better practice, on account of the marked depression which occasionally comes on even when least expected, whatever drug is employed, to give small doses repeated as often as necessary, rather than to attempt to expel all the parasites in one or two doses. Oil of chenopodium has not yet been tried, because it was unavailable when called for. Restricted diet and saline purgatives both before and after are of course indicated. Dead flukes will usually begin to be passed within twelve hours after taking the anthelmintic, and will continue to come for two or three days. Tonics are rarely needed, as it is astonishing how rapidly the anemia disappears once the intestines are cleared.

Prophylaxis, naturally, will consist in the avoidance of uncooked food. The life history of this fluke is still to be worked out, but the fact that fresh water snails enter largely into the diet of the people of this region, and that before being eaten they are subjected to only slight scalding, is suggestive that these molluscs may be the secondary host. Shrimps have also been considered, but they are in general quite thoroughly cooked.

THE PARASITE

The parasite in question exhibits such wide variations in morphology that four species have been described, and recently Brown has suggested a reclassification into two groups differentiated chiefly by the presence or absence of cuticular spines. The literature on the subject is somewhat contradictory and confusing, and until recently the material has been so scanty and appeared at such great intervals of space and time, that a restudy of the subject in the light of considerable clinical experience and with a larger amount of laboratory material seemed likely to yield important results. The following report, which is preliminary to further studies on the life history of the parasite and its effects on man from the pathological standpoint, is based on a

practice extending over several years in a heavily infested district (Shaohing, China), supplemented by a laboratory study of 433 specimens. Twenty-one flukes have been cleared, the ventral sucker with cirrus sac and metraterm attached have been dissected out from three, ova have been removed for measurement from the lower uterus of nine, and seventeen series of microscopic sections have been made of individuals conforming to the descriptions of the three types which have been described as *F. buski*, *F. rathouisi* and *F. goddardi*, including five serial sections in different planes.

In selecting specimens as representatives of each type, in general, those were chosen which in size fell within the published measurements; viz. a length of 25 mm. or more for *F. buski*; 21-24 mm. for *F. goddardi*, and 15-20 mm. for *F. rathouisi*. But a few exceptions were made where other physical features clearly indicated a different classification. This fact will explain the apparent inconsistencies in the grouping, and illustrate at the same time the extreme difficulty encountered in making any distinctions on account of the many borderline cases.

In summarizing the results of this study, it will be convenient to consider first the variations upon which differentiation into species has been based; and to conclude with a somewhat detailed description of the morphology as a whole, especially of those features about which there has hitherto been doubt.

The variations which have figured in the differentiation into separate species may be grouped as follows:

1. *Color and Consistency*.—Specimens preserved in alcohol vary in color from brown to grayish-white, and frequently the vitellaria are clearly outlined by a bluish black pigmentation along the lateral and posterior margins (Pl. XII, A). Some are firm in consistency, while others are flabby and soft. These variations are found in individuals of all the types of this genus which I have examined, and are undoubtedly due to postmortem changes. The fluke when alive or freshly killed is of a deep pink color, not unlike that of boiled ham, and the great majority of all flukes recovered from the stools are of this color and are firm in consistency. Occasionally flukes are passed which are pearly white with dark borders, and are flabby in appearance, and to the touch. Under the microscope such specimens are seen to have lost their cuticula (Pl. XIV, A), and the cells of the yolk glands have fallen away from the basement membrane of the acini and are collected in a more or less disintegrated mass in the lumen, the nuclei being deeply pigmented. Recalling the fact that flukes are often two or three days in the intestine after the anthelmintic has been taken, it is easy to understand that partial digestion of the surface has occurred, quite sufficient to account for the phenomena observed. Similar but

less marked changes occur in specimens allowed to remain for a considerable time in water before being placed in preservative.

2. *Size and Shape*.—Variations in size and shape are so extreme (Pl. XII, A) as to warrant a belief in the existence of more than one species until it is found that gradations from one to another type are so gradual as to make lines of demarcation quite impossible.

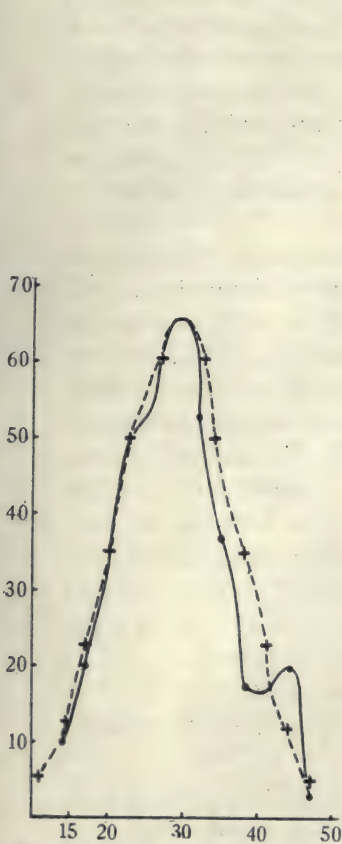


Fig. 1. Length frequency curve constructed from measurements of 378 individual flukes.

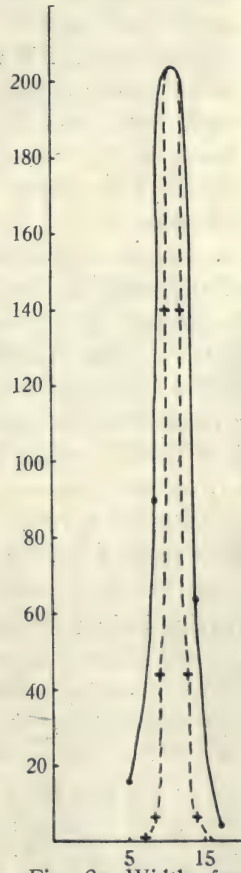


Fig. 2. Width frequency curve.

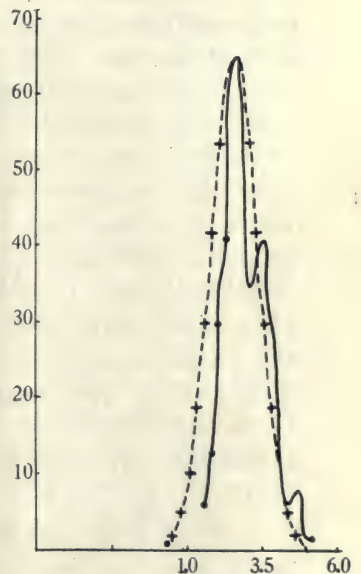


Fig. 3. Proportion frequency curve, i. e., ratio of length to width.

In this investigation measurements were made of 378 flukes ranging from 13 to 48 mm. in length and from 4 to 17 mm. in width. Progression in length and width measured in millimeters, and in the ratios between them, measured in $\frac{5}{100}$ of the width was found to be perfectly even. Frequency curves were then plotted and are shown in Graphs 1, 2 and 3. A certain amount of error is possible here, due to the fact that the flukes were not all in the same state of muscular contraction when fixed, but all the curves, for length, for width, and

for proportion, are so distinctly unimodal, and conform so closely to the corresponding theoretical curves for variations* as to leave little doubt that we are dealing with a single species.

The thickness measured at the middle varies in this series from 0.8 to 3.0 mm., while over the acetabulum, the thickest part, the maximum found was 3.5 mm., and the variation is somewhat less. No relation can be found between the thickness and the various suggested species. That described as *F. rathouisi* is characteristically "short and stocky"—one such measured 20x12x2.8, was brown in color, firm in texture, and in a state of opisthotonos. But another measures 19x13x2.0, is glistening white with dark borders, and lies flat and relaxed. On the other hand, a fluke (*e* in Pl. I, *A*) typical of *F. buski*, measured 43x12x1.0, but others measuring 40x9x2.3 or 32x10x2.3 or 33x10x3.0 also occur.

In part, these differences may be ascribed to natural variation, but some are certainly due to the state of muscular contraction at the time of fixation. This is apparent not only from the numerous and deep transverse rugae which are seen frequently in preserved specimens (cf. *i* and *j* in Pl. XII, *A*), but also from observations on flukes in the fresh state. In one instance 22 flukes were brought in immediately after evacuation, all of which appeared to be long and narrow (about 35x8 mm.) and many of which showed an inrolling of the edges of the cephalic portion about the long axis so as to form almost a complete sheath. I was interrupted in taking their measurements, and after remaining in water four hours all were found to have assumed the ordinary appearance, varying in length from 18 to 35 mm., in width from 13 to 20, the average being 28.6x16.4, and extremes being 35x20, 18x16 and 28x13. On another occasion a fluke measuring 28x19 on evacuation, was found after standing two days in water to measure 41x17.

3. *Head Cone*.—In none of the Shaohing flukes is there a distinct "head cone" or shoulder such as occurs in *Fasciola hepatica*, for example; but in certain specimens (cf. *i*, *g*, *f* in Pl. XII, *A*) there is a narrowing at the level of the genital pore, and when viewed from the side a posterior bulging. This is due to the contraction of the dermo-muscular tube and parenchymal muscles closely about the solid muscular bodies of the region (oral sucker, pharynx and acetabulum), and is not distinctive of any of the three types.

* The normal curve in all these figures is plotted according to the commonly used formula

$$y = \frac{n}{\sigma \sqrt{2\pi}} \cdot \frac{1}{e^{\frac{x^2}{2\sigma^2}}}$$

4. *The Cuticula*.—In the early description of *F. buski* by Cobbold, the integument is said to be "smooth and unarmed," and Odhner, Ward, Looss, Braun and Rodenwaldt either state that spines are absent or adopt the view generally held that they are absent. Heanley and Jefferys state positively that they are present "though very difficult to find in some mounted specimens" (Heanley), and Leiper says they are present in the species *F. rathouisi* and *goddardi*, and explains their occasional absence from certain specimens by the "deciduous character" of the cuticula, claiming to have seen in a section of Rodenwaldt's specimen the regularly recurring pits in the cuticula from which the spines have dropped out. More recently Brown has found an apparent coincidence between the presence or absence of spines and the marked differences in gross appearance already discussed, and suggests a reclassification of the several varieties now named into two groups on the basis of this characteristic.

Of the present series of 433 flukes, half (including all the variant types illustrated in Pl. XII, *A*, and among them typical examples of the two groups suggested by Brown), were examined by strong reflected and transmitted light, and in all of these spines were found though in many cases only with difficulty and in very small numbers, due to the deciduous character of the cuticula to which Leiper has already called attention.

Of the flukes photographed in Plate XII, *A*, *e*, *f*, *j* and *k* have since been imbedded and sectioned. In *j* and *k* the cuticle is well preserved and the spines numerous; in *e* and *f*, on the contrary, the cuticle, except in a very few scattered spots, is entirely gone. A comparison of Plate XII, *B*, Plate XIII and Plate XVII, *A*, shows how readily even in fairly well preserved specimens the cuticula strips off from the basement membrane carrying the spines with it. These facts furnish a satisfactory explanation of the occasional failures hitherto to demonstrate the presence of cuticular spines, and warrant the conclusion that they are charactersitic of the entire genus.

5. *Form and Size of Various Viscera*.—The cirrus sac is convoluted in all specimens, varying from two or three close turns like the strands of a rope to larger spirals, and its course may be straight or sharply bent upon itself; but neither these variations nor the relative length of the sac posterior to the acetabulum are characteristic of any group, being found in each. The diverticulum of the seminal vesicle, the so-called "cecal appendage" was present in all specimens examined.

The shellgland is an elastic body, and though normally ovoid in shape, is capable of being markedly affected by the surrounding muscles; appearing in one highly contracted specimen (flake *m*) as an antero-posteriorly flattened disk extending the entire dorsoventral diameter of the body.

TABLE 1.—VISCERAL MEASUREMENTS IN MICRONS. LETTERED INDIVIDUALS REPRESENT SECTIONS; THE OTHERS CLEARED FLUKES

Flukes: Cleared and Sectioned	Oral Sucker		Pharynx	Genital Pore	Ventral Sucker			Shell Gland			Cirrus Sac*		Testes	Vitel- line Acini	Remarks
	Whole	Or- fice			Whole Organ	Orifice	Dis- tance from Head	Size	Dis- tance from V. S.	Dis- tance from Head	In Microns	Per Cent.			
F. buski	45 × 13 × 1.3	876 × 876	350	2,312 × 2,550	876 × 1,518	1,460	1,401 × 1,576	6,424	15 mm.	3,796	50	292-759	58-175	
	33 × 8	350 × 467	175	116 × 700	2,511 × 2,102	1,051 × 1,216	1,752	992 × 1,168	7,884	12 mm.	4,672	59	? -642	58-146	
	29 × 11	408 × 584	280	294 × 408	2,920 × ?	876 × 1,460	1,284	1,109 × 1,226	7,416	12 mm.	4,204	54	116-700	58-116	Cirrus sac very angular course
	27 × 9	175 × 408	2,336 × 2,336	467 × 584	1,284	934 × 1,168	6,716	12 mm.	3,212	47	116-525	58-175	
	29 × 13	700 × 817	408	584 × 700	2,628 × 2,236	1,576	1,576	11 mm.	4,672	76(?)	116-525	58-120	
	39 × 11	700 × 876	350	292 × 584	3,212 × 1,752	1,284 × 1,343	1,868	1,343 × 1,460	9,928	16 mm.	4,555	45	116-700	58-175	
	31 × 14 × 2.3	700 × 700	467	584 × 759	2,336 × 2,044	876 × 1,168	1,868	642 × 876	7,884	13 mm.	4,380	55	175-584	58-116	
	35 × 9 A	430 × 619	378	292 × 516	2,752 × 2,150	1,204 × 1,720	7,740	5,160	67	
	43 × 12 E	602 × 619	326	326 × 430	2,924 × 2,201	1,548 × 1,548	9,718	5,160	53	Slightly irregular rounded Sagittal section
	16 × ? P	430 × 240	* 86 × 172	1,874 × 1,100	860 × 820	4,644	2,408	52	
F. rath.	40 × 9 × 2 N	430 × 550	292	498 × 567	2,854 × 1,823	946 × 1,376	8,944	4,816	54	
	15 × 10	58 × 292	* 408 × 467	2,336 × 1,752	584 × 1,460	700 × 876	7 mm.	175-408	Cirrus protruding
	21 × 11	292 × 350	116 × 350	292 × 1,284	759 × 1,168	4,263	9 mm.	58-175	
	19 × 11	595 × 750	292	642 × 817	? × 2,044	876 × 1,401	4,558	2,404	56	58-116	
	16 × 7 L	244 × 261	120	309 × 447	1,892 × 1,513	516 × 688	4,558	2,404	83	Sagittal section, greatly contracted
F. goddardi	20 × 12 M	344 × 258	...	464 × 430	2,476 × 1,462	946 × 1,720	4,888	4,128	83	
	22 × 10	467 × 525	292	2,452 × 2,044	525 × 1,168	1,168	1,168 × 1,284	4,672	9.5 mm.	2,628	56	? -584	58-175	
	23 × 10	580 × 580	292	233 × 467	2,511 × 2,044	642 × 1,401	1,051	1,460 × 1,460	5,256	11 mm.	3,679	69	? 408	58-116	
	22 × 11	584 × 700	292	2,044 × 1,635	817 × 1,169	1,284	876 × 876	6,716	10 mm.	55	116-350	58-116	
	22 × 9	2,336 × 1,752	580 × 700	1,284	934 × 1,343	6,716	10 mm.	3,796	
	20 × 6 B	2,201 × 1,444	6,192	2,408	40	
	23 × 9 C	412 × 412	240	619 × 636	2,580 × 1,806	946 × 1,290	7,098	3,668	51	Cirrus protruding
	23 × ? H ₁	447 × 412	86	395 × 567	2,322 × 1,462	946 × 1,118	5,160	3,440	66	Sagittal section, cirrus protruding
	23 × 8 H ₂	533 × 602	344	* 154 × 516	2,081 × 1,840	1,118 × 1,118	4,472	3,010	68	Slightly heart shaped
	25 × 8 J	688 × 516	103	584 × 705	2,958 × 1,720	946 × 1,032	4,128	1,892	47	Sagittal section, contracted
F. goddardi	23 × 10 K	464 × 447	2,150 × 1,462	1,118 × 1,238	7,740	3,612	46	
	20 × 6 O	378 × 430	223	* 154 × 240	2,304 × 1,892	946 × 1,204	4,816	2,408	50	

* First column is length of sac posterior to acetabulum; second column is ratio of this length to entire distance between acetabulum and shell gland.

Measurements of the various organs are tabulated in Table 1, from which it appears that the variations which occur are not characteristic of the groups, but rather bear a general relation to the size and development of the individual.

6. *The Ova*.—Observation of the ova in hundreds of samples of fresh feces leaves an impression on one of their essential unity, an

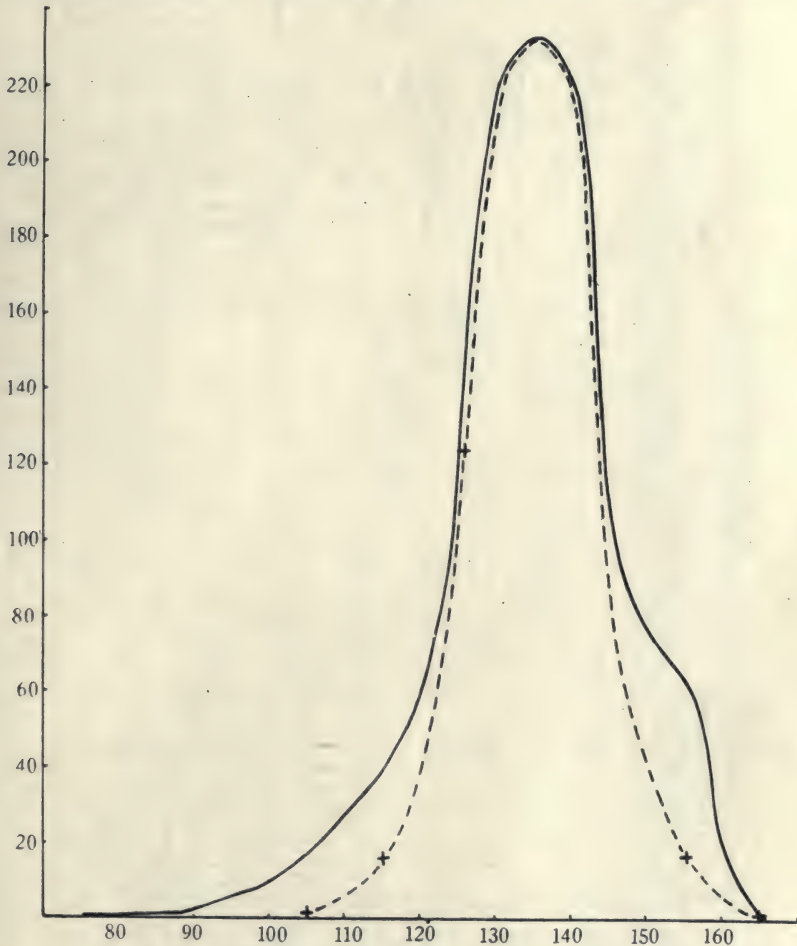


Fig. 4. Curve representing length of ova taken from entire series.

impression confirmed by the present series of measurements. In all, 576 eggs were measured, of which 150 were from two separate samples of fresh feces, and the rest from the lower uteri of nine preserved flukes, including representatives of each variety. The entire series is found to be grouped, in length, about the single mode 130-140 μ with variations conforming fairly well to the normal curve (Graph 4). The same mode, it will be noted, obtains for each of the groups as well,

TABLE 2.—TABLE OF MEASUREMENTS OF OVA

Source	No.	Length in Microns										Width in Microns										Remarks	
		71/ 80	81/ 90	91/ 100	101/ 110	111/ 120	121/ 130	131/ 140	141/ 150	151/ 160	161/ 170	51/ 55	56/ 60	61/ 65	66/ 70	71/ 75	76/ 80	81/ 85	86/ 90	91/ 95	96/ 100		101/ 105
F. buski 1. 40×11..... 2. 27×9.....	79 28	21 16	58 10	4 0	9 0	42 2	12 0	6 0	6 0	6 1
Group b.....	2	37	68	2	7	16	4	9	44	12	6	7	
F. rathousi 3. 17×5..... 4. 13×8..... 5. 17×9..... 6. 22×13..... Group r.....	30 30 30 100	8 14 3	18 16 12	1 1 2	5 6 9	3 10 50	5 0 41	5 2 17	1 1 2	
F. goldardi 7. 25×9..... 8. 24×12..... 9. 23×12.....	30 50 49	1	1	5	7 1	2 1	1	2 24 8	3 19 14	4 4 7	4 4 ...	4 ...	2 ...	
Group g.....	...	1	1	5	14 7	3	11	36	50	1	1	...	1	34 36	27	20	4	4	2	
Feces 1. Feb. 1909..... 2. Sept. 1917..... Group f.....	50 100	1	...	5 14 6	28 64 92	13 14 27	2 2 4	4 1 5	11 65 76	...	2 8 2	...	2	...	
Total.....	576	1	1	6	19	40	106	237	101	64	1	3	16	19	222	149	61	68	16	9	
Series 2.....	497	1	5	33	104	233	89	32	2	16	19	212	132	37	50	12	7	
Group 2 Nos. 1, 2, 3..... Nos. 4, 5, 6, 9.....	1 1 ...	1 5 ...	5 21 ..	17 17 30	57 30 77	68 77	2 1	7 4	10 6	28 118	12 83	18 35	54 12	16	9	

except Group G, in which it is raised somewhat by the abnormality discussed below. If now on account of the great divergence shown by individuals in this group and for other reasons we throw out *F. goddardi* altogether, and make but two species as shown in Group 2 of the table (Table 2), here again both species—*buski* and *rathouisi*—

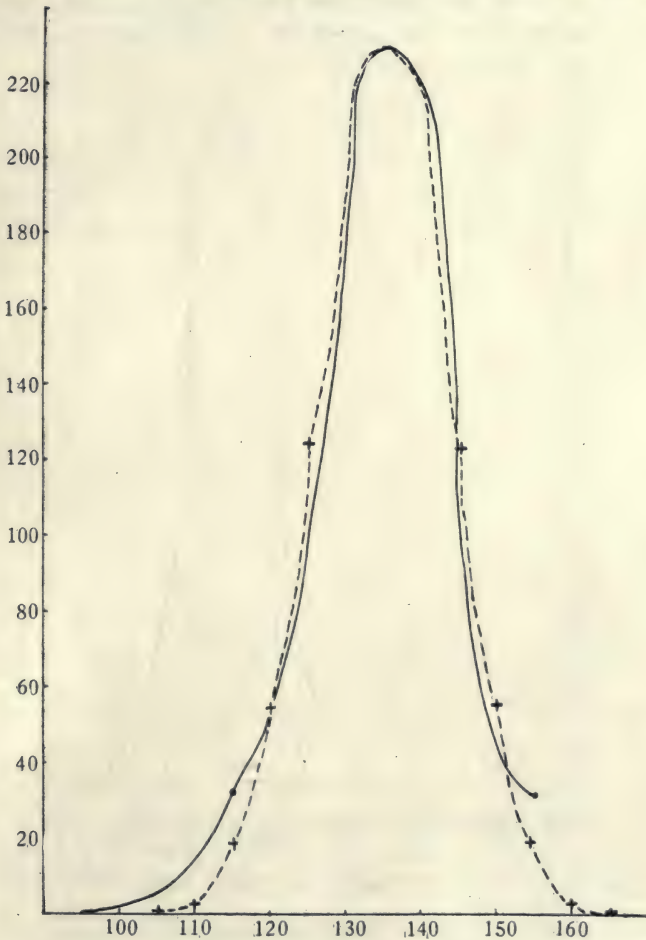


Fig. 5. Curve representing length of ova in amended series.

are found to have the same mode. It is also noteworthy that eggs deposited in feces—a normal condition—form a curve which agrees very closely both with its own normal and with the curve for the entire series, and has a much smaller standard deviation—spreads less—than do any of the curves for eggs from an individual. Graphs 4-7 graphically represent this fact, which is strikingly apparent under the microscope, viz., that ova taken from the body of the parasite present

far greater variations in contour and in size than are seen under normal conditions. In particular, such variations in contour were especially noticeable in eggs from fluke 9, and a large proportion of them were apparently swollen; i. e., a clear space appeared between the yolk contents and the shell, the former remaining normal in size and shape, while the shell measured from $23\text{--}27\mu$ more in length, and about 11 more in width. To a less extent these conditions were found also

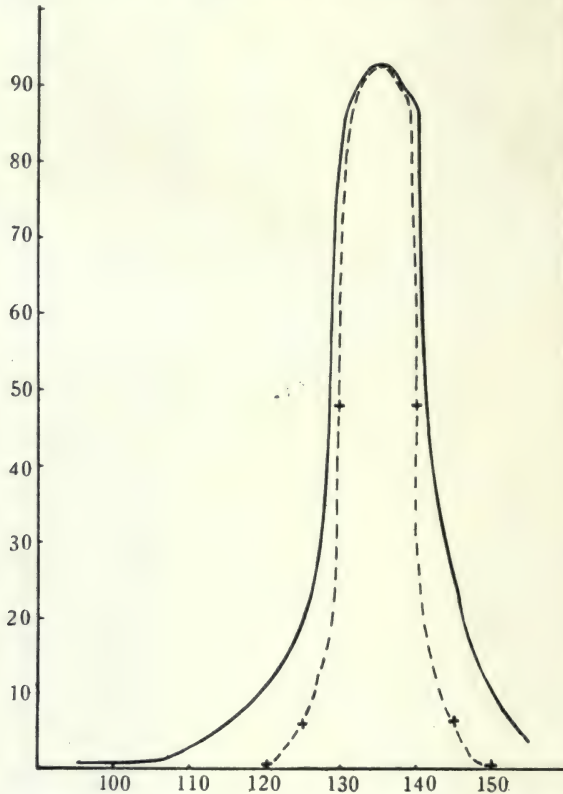


Fig. 6. Curve representing length of ova taken from feces.

in flukes 2, 3 and 5, and are doubtless abnormalities, as I have never seen them in feces specimens. On the other hand, eggs from fluke 7 were relatively so few (i. e., the total number obtained from the uterus were so few), and are grouped about a mode so much smaller than the average that they may fairly be considered immature, especially since in another fluke of about the same dimensions practically no eggs were found. If now on the supposition that they are abnormal, the eggs from these two flukes, 7 and 9, be excluded from the series, and the curve of the remainder be plotted as is done in Graph 5, the close correspondence between observed and theoretical curves furnishes

conclusive evidence of the correctness of the hypothesis. Further, that these variations are individual abnormalities rather than indications of specific differences seems clear from the fact that in the entire series they stand alone, that the flukes from which they were taken are in other respects indistinguishable from each other and from flukes yielding normal eggs, and that the eggs differ as described above from any that I have seen that were deposited in the normal manner. In the above discussion the short diameter of the egg has not been considered because it does not alter the problem. Those measurements, however, will all be found in the table (Table 2).

Writing in the Encyclopaedia Britannica, Peter Chalmers Mitchell defines a species as "an assemblage of organic forms which . . . if they differ among themselves differ less markedly than they do from

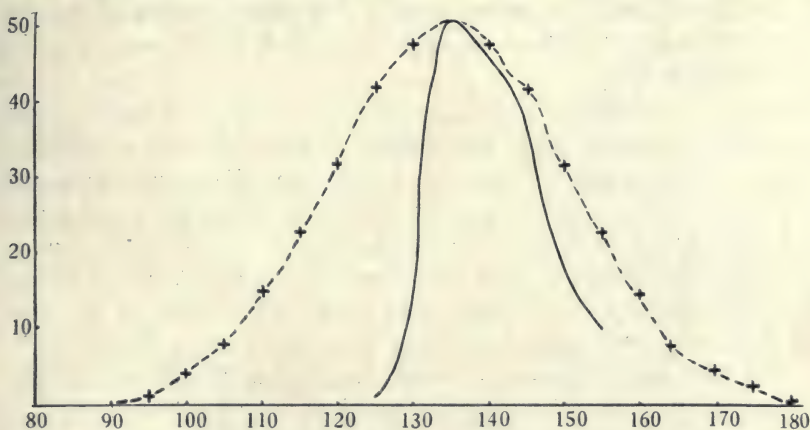


Fig. 7. Curve representing length of ova taken from fluke No. 6.
For further details compare text.

those outside the species, or if differing markedly are linked by intermediate forms." With such a definition and in the light of the foregoing discussion it would seem that the three varieties represented in the Shaohing specimens should be regarded as belonging to but one species, *F. buski*. Poirier's original specimen on which the species *rathousi* was based, came from a suburb of Shanghai, the commercial metropolis of the region, to which people from neighboring cities have flocked in large numbers. In recent years a few cases of fasciolopsis infection have been reported from Shanghai, and some of them at least are known to have been natives of Shaohing. It is therefore quite probable that Poirier's specimen came from Shaohing, which if true would tend to support the argument from morphology presented by Odhner and others to the effect that it was in reality but a contracted form of *F. buski*. Kwan's fluke has been conclusively shown by Leiper to be a mutilated specimen of the same species, and the

description of *F. fülleborni*, given by Rodenwaldt, agrees with that of *F. buski*, as modified by the present study in every particular except that it is said not to possess cuticular spines nor the characteristic diverticulum of the seminal vesicle. If Leiper's statement, already quoted, regarding spines in this case be accepted, there remains but a single point of difference outstanding; and in view of the limited amount of material (three specimens from a single case) on which the description was based, further study would seem to be required before the identity of this as a separate species can be accepted.

MORPHOLOGY OF THE PARASITE

Assuming then the existence of but a single species in the Shaohing flukes, the following account of its morphology may be given, which applies equally to all varieties, except that measurements of internal parts are to be understood as applying to individuals approximating the average in size.

General Appearance.—*F. buski* is a flat, elongated fluke, typical specimens measuring about 30x12x2 mm. Busk reported a maximum length of three inches (75 mm.?). In Shaohing the greatest measured length was 55 mm., and the greatest width 20. Immature forms 5 mm. or less in length have been recovered from feces, and in six uncontracted forms measuring from 9 to 21 mm., but a few poorly developed eggs were found in the longest, and in the others none at all, from which the minimum adult length may be assumed to be 20 mm.

The head end is somewhat pointed, with a poorly defined shoulder, the tail bluntly rounded, the lateral margins even or slightly wavy. In fresh specimens the color is a uniform deep pink—ham color—and to the touch and the unaided eye the integument appears smooth with transverse lines more or less prominent according to the state of muscular contraction.

The Cuticula.—Microscopically, this is seen to consist of a basement membrane overlaid with the true cuticula, which under high magnification shows fine striae vertical to the plane of the surface. It is somewhat thicker on the dorsal (19 to 34 μ) than on the ventral surface (15-27), the maximum being at the level of the upper border of the acetabulum, from which point it becomes thinner in both directions. True cuticula similar to that covering the surface of the body, but thinner, extends for varying distances into all the organs which open externally.

The entire ventral surface, including the narrow strip anterior to the oral sucker, is armed with spines, which are most numerous in the acetabular region (Pl. XVII, A). These spines are deeply imbedded in the cuticula, their bases resting on or in the basement membrane,

and are directed caudad at an angle of 30-45 degrees, the merest tip projecting beyond the surface. They are arranged, somewhat irregularly, in alternating transverse rows (Pl. XIII, *B*) which in the most thickly covered regions may be almost in contact, but will average 10 to 15 μ apart, and toward the tail may be separated by 160 μ or more. Similarly, in the transverse direction the spines are practically in contact over the acetabulum, but toward the tail the interval between the bases of adjoining spines varies from 19 to 40 μ .

The Spines.—The spine itself (Pl. XIII) is a scale-like structure, 25 to 30 μ wide at the base, and 30 to 34 μ long. The sides converge a trifle toward the tip which is bluntly rounded and curved backward. The upper surface of the spine is flat or, especially toward the tip, slightly concave. In the lower surface the curvature is somewhat greater, giving to a transverse section a crescentic outline. The body of the scale increases in thickness from a thin edge at the tip to 10 to 13 μ at the base where it flares out rapidly like the thorn of a rose. The surface of the base, where it is attached to the basement membrane, presents two, three or rarely four cusps, causing a transverse section through it to appear like a group of rounded or irregular bodies. The size of the scales varies with the stage of development of the parasite, and with the location, being smallest around the oral sucker and the genital pore. Extremes in length were 22.8 and 41.8 μ , other dimensions being in proportion.

The Musculature.—Muscular fibers occur in the walls of the various viscera, and are particularly well developed in the cirrus and the metraterm. In addition, the muscular system comprises the following:

1. *The dermo-muscular tube* lies immediately beneath the basement membrane of the cuticula, and consists of annular, oblique and longitudinal fibers. At rather frequent intervals are found other longitudinal and also dorso-ventral fibers (the parenchymal muscles), and certain fibers attaching various viscera, especially the two suckers and the shell gland to the dermo-muscular tube.

2. *The oral sucker* is situated at the anterior extremity, its orifice being sub-terminal on the ventral surface, and its long axis, which is continuous with that of the pre-pharynx and pharynx, being oblique to the surface (Pl. XII, *B*). In younger specimens the sucker is nearly globular, but with age the transverse diameter becomes longer, and the dorso-ventral shorter relative to the longitudinal. In well developed specimens these diameters will range from about 0.5 to 0.7 mm. The oral orifice is normally circular, 0.3-0.4 mm. in diameter. The entire organ (and this applies as well to the pharynx and the ventral sucker) is enclosed in a capsule and suspended by means of several processes

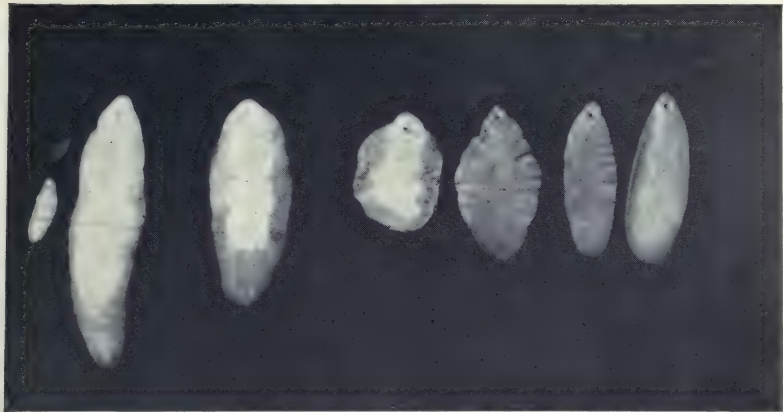
of muscular and connective tissue in a sinus of the excretory system, doubtless in order to facilitate muscular contractions. In addition to this, considerable motion in its longitudinal axis, whereby the organ may be partially extruded through the oral ring, is made possible by its free attachment to the ring by means of an eversible collar of cuticula and connective tissue, and by the interposition of a collapsible portion of the alimentary tract—the pre-pharynx—between the oral sucker and the pharynx. Whether this freedom of motion is used for locomotion as well as for feeding is not determined, but seems probable.

2. *The pre-pharynx*, whose function seems to be to permit motion of the oral sucker, is surrounded by a well developed muscle consisting of circular fibers, and extending from the upper surface of the pharynx to about the middle of the oral sucker, which it is thus able powerfully to reinforce (Pl. XII, B).

3. *The pharynx*, which surrounds the alimentary tract just prior to its bifurcation and lies beneath the dermo-muscular tube posteriorly, is a spheroidal mass of radial and circular fibers measuring from 0.40 to 0.75 mm. antero-posteriorly, and from 0.70 to 0.99 mm. transversely.

4. *The ventral sucker* is a powerfully developed, bell-shaped organ, situated near the anterior extremity (1.2 to 1.8 mm. from the tip) and so placed that its long diameter and the plane of its orifice are oblique to the ventral surface, the anterior lip of the orifice being somewhat longer than the posterior. Its total length varies from 2.3 to 3.2 mm., and its greatest diameter from 1.7 to 2.5 mm., the dorso-ventral diameter being somewhat shorter than the transverse. The orifice, normally circular with a diameter of 1.0 to 1.5 mm., is in preserved specimens often transversely elliptical or irregularly heart-shaped. The acetabular cavity is lined throughout with an extension of the cuticula.

The alimentary tract extends as a single tube from the oral aperture to just beyond the pharynx where it bifurcates, forming the two ceca which proceed laterally to the outer margins of the acetabulum, at which points they bend sharply caudad and follow a sinuous course to the posterior extremity, ending blindly near each other. This course is marked by two main inward curves, viz., at the level of the shell gland, and between the testes; but the ceca follow the outline of the viscera they enclose and hence the number and degree of their curves depend in part upon the development of the individual, and the state of its muscular contraction. The lumen of this tract varies considerably in size. At the oral aperture it appears in sections as a transverse slit, 0.2 to 0.3 mm. by 0.085 to 0.100 mm., but immediately expands, then narrows, to expand again within the pharynx, from



A *e* *f* *g* *i* *j* *k*



B

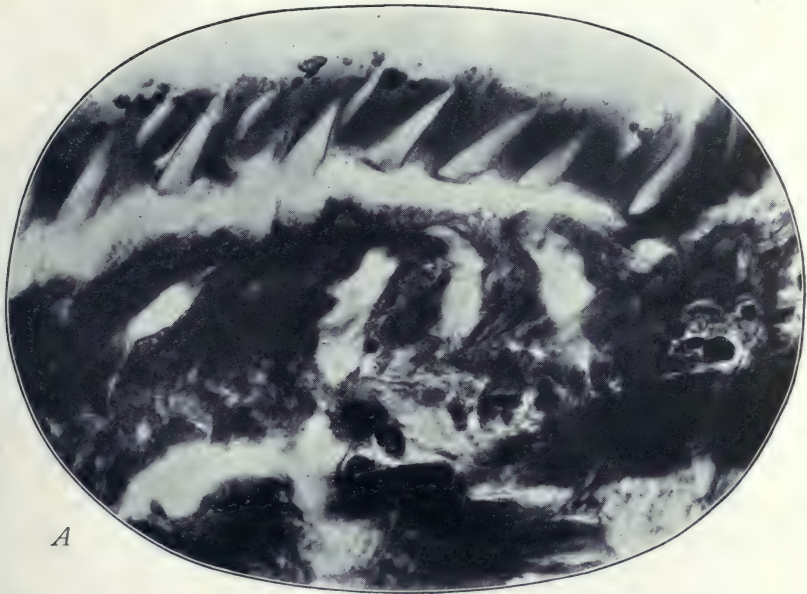
PLATE XII

EXPLANATION OF PLATE

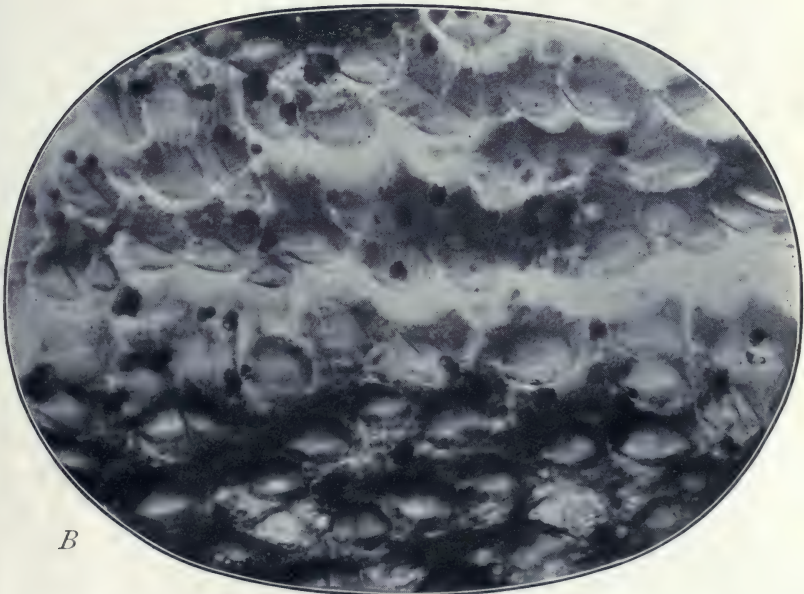
A. Varieties of the fluke encountered in Shaohing.

B. Anterior extremity, showing especially the sinuses surrounding oral sucker, pharynx, and ventral sucker; nerve cell in pharynx; cuticula stripping off and also lining of oral and ventral suckers. Sagittal section. ($\times 68$.)





A



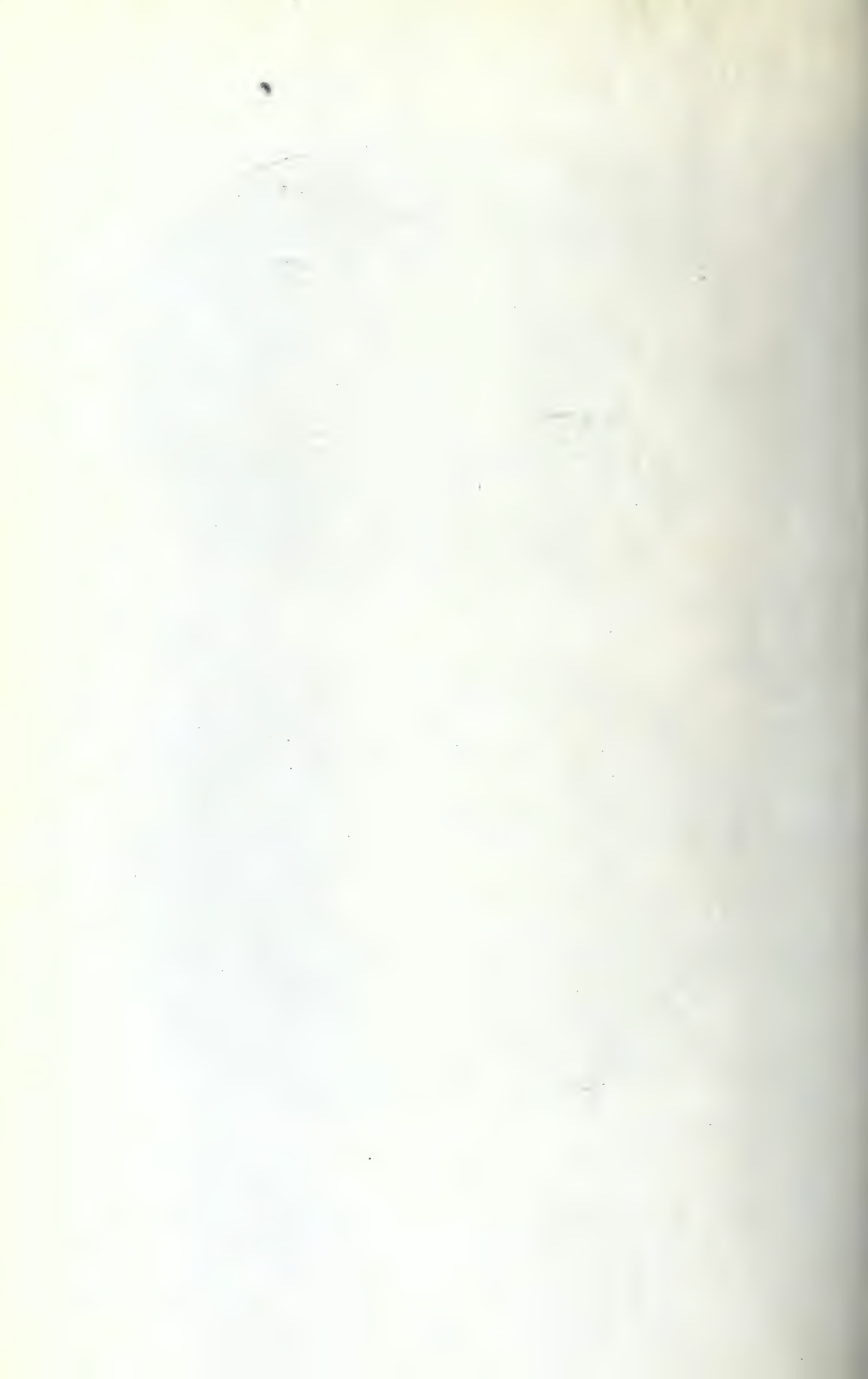
B

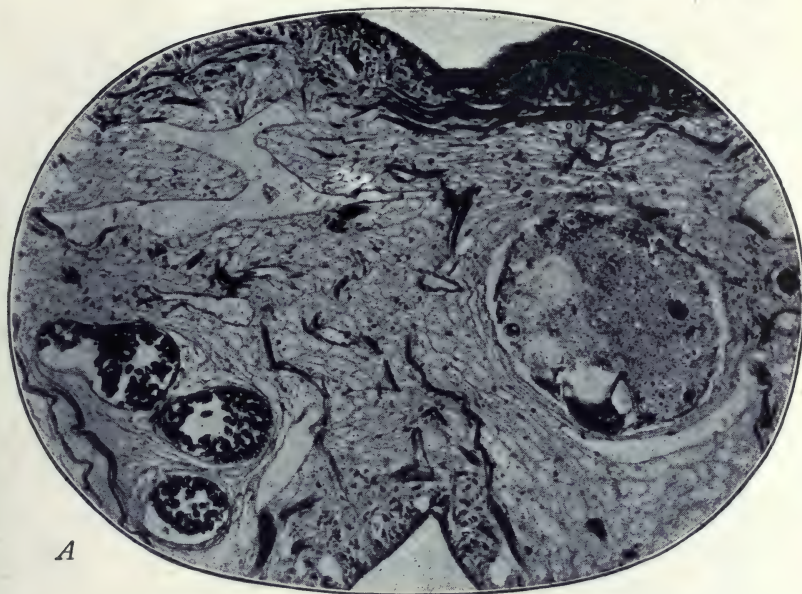
PLATE XIII.

EXPLANATION OF PLATE

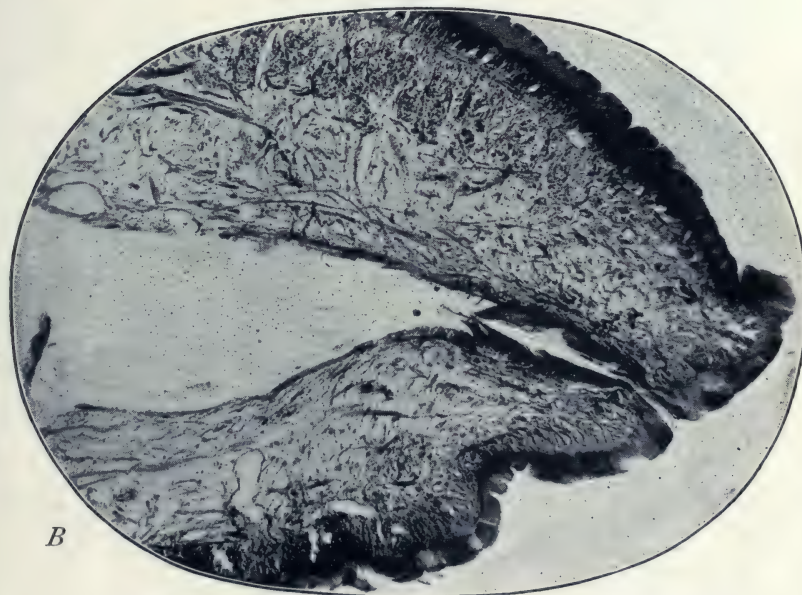
A. Spines embedded in cuticle, and being stripped off with it. Sagittal section. ($\times 450$.)

B. Ventral spines, arranged in alternate rows. Section in ventral plane. ($\times 450$.)





A



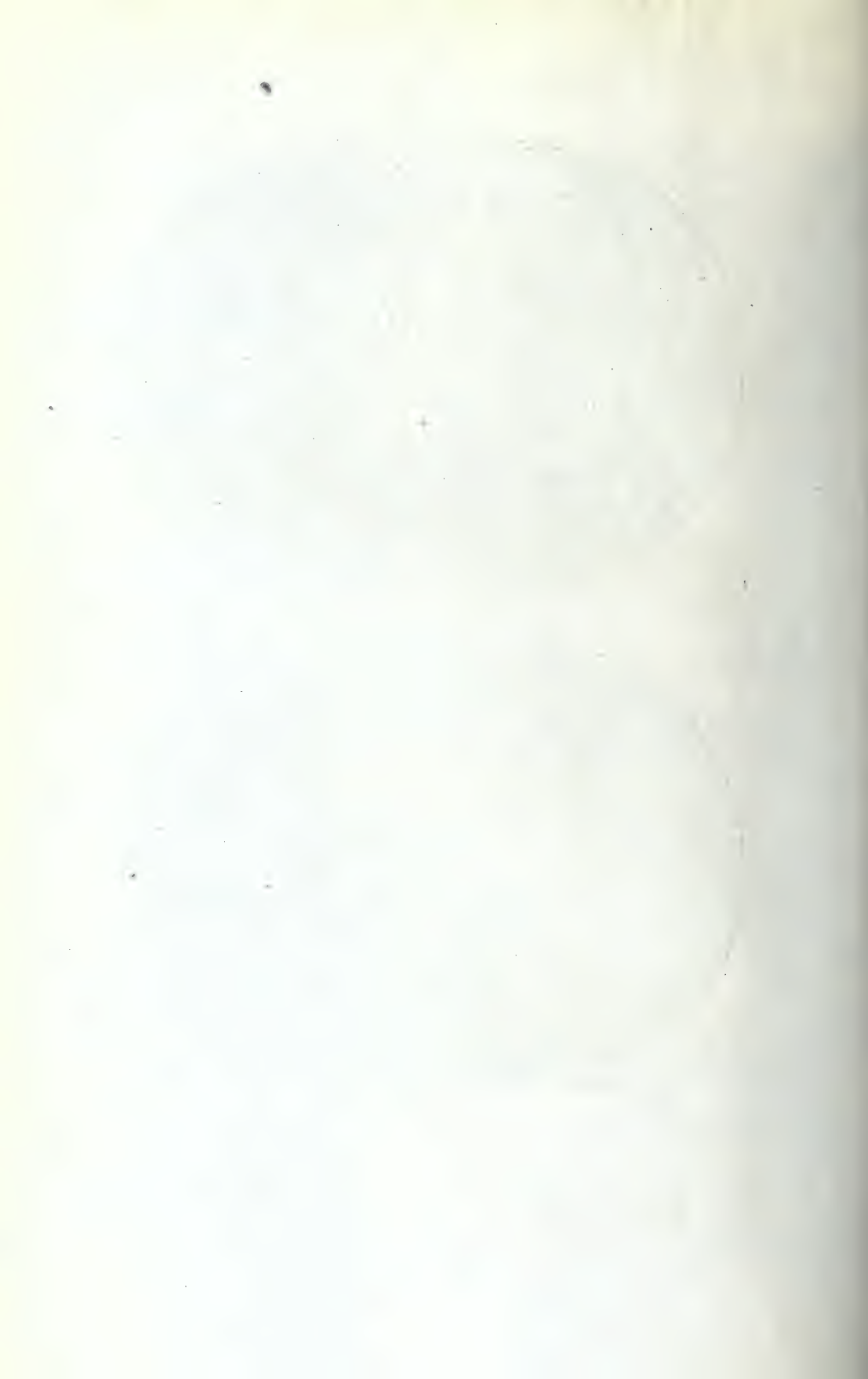
B

PLATE XIV

EXPLANATION OF PLATE

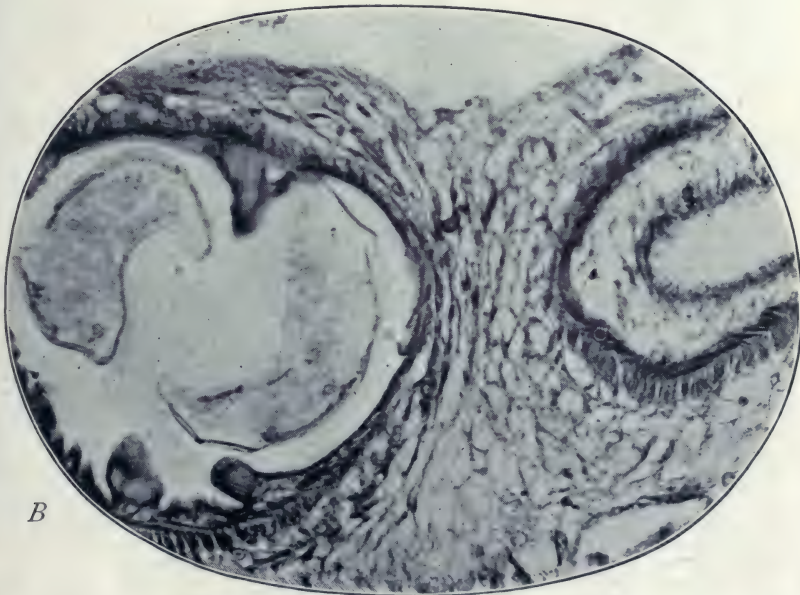
A. From fluke F showing entire absence of cuticula. Also coils of ovary at left, nerve cell at right. Sagittal section. ($\times 68$.)

B. Posterior extremity, showing excretory vesicle and duct. Sagittal section. ($\times 68$.)





A



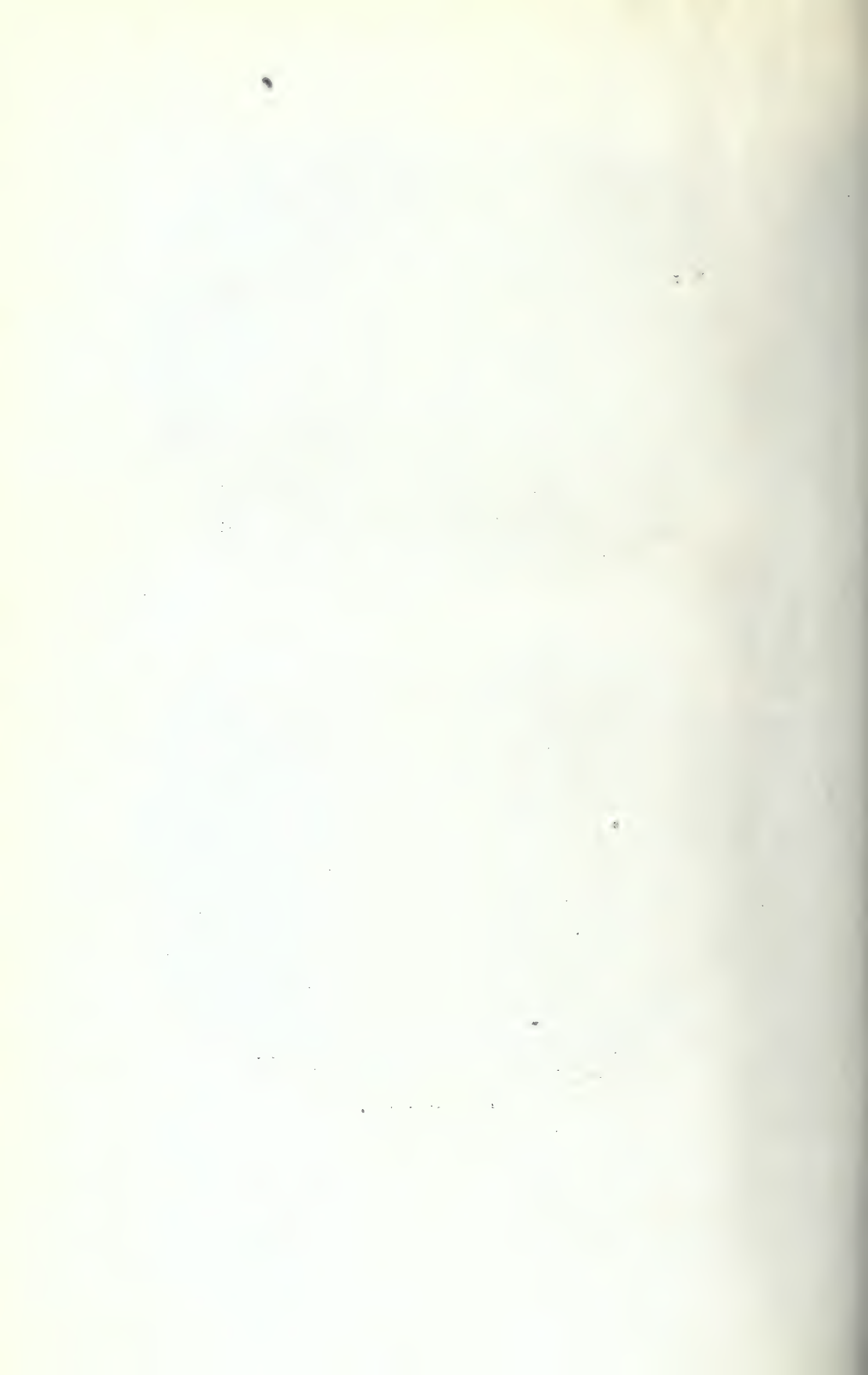
B

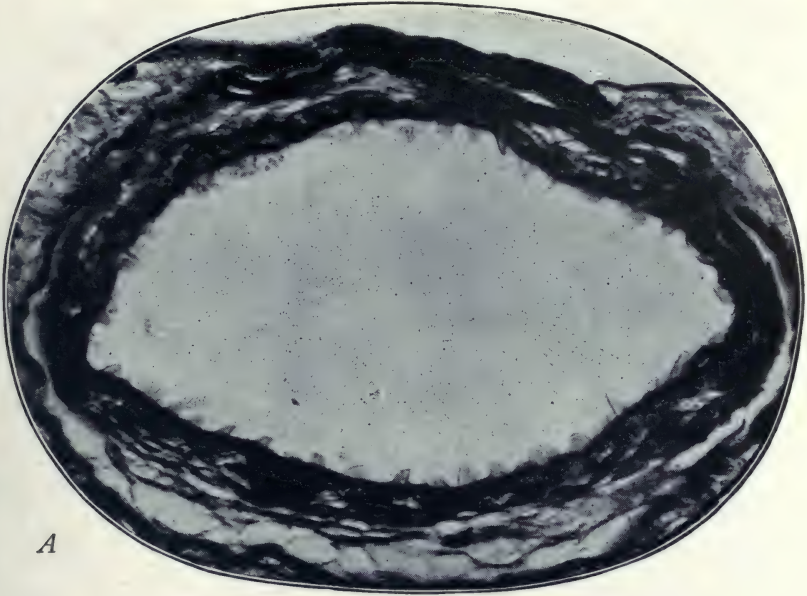
PLATE XV

EXPLANATION OF PLATE

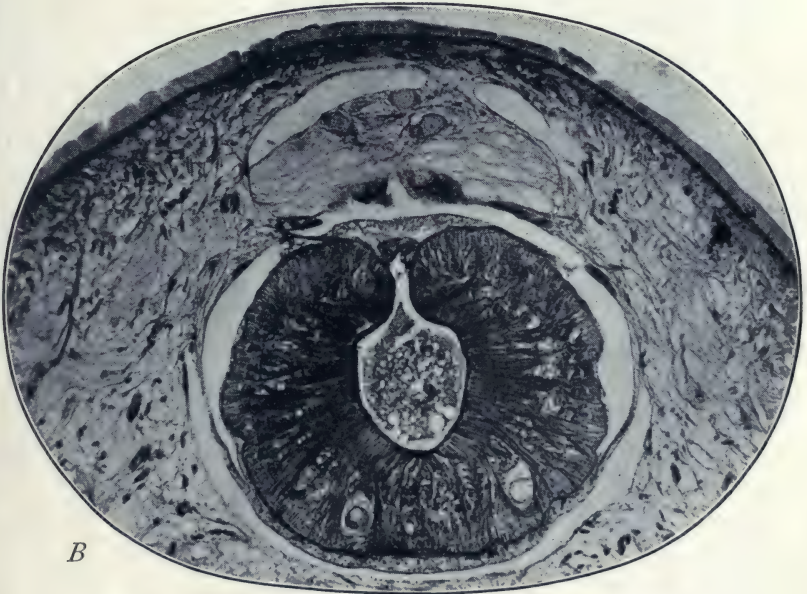
A. Shell gland, with vas efferens at left, coils of Laurer's canal above, junction of common yolk duct with oötype in center, and vitelline receptacle below. Ventral section. ($\times 90$.)

B. Metraterm and everted cirrus just below level of genital pore, showing 3 large spines in metraterm, also spines of cirrus and pre-cirral canal. Ventral section. ($\times 360$.)





A



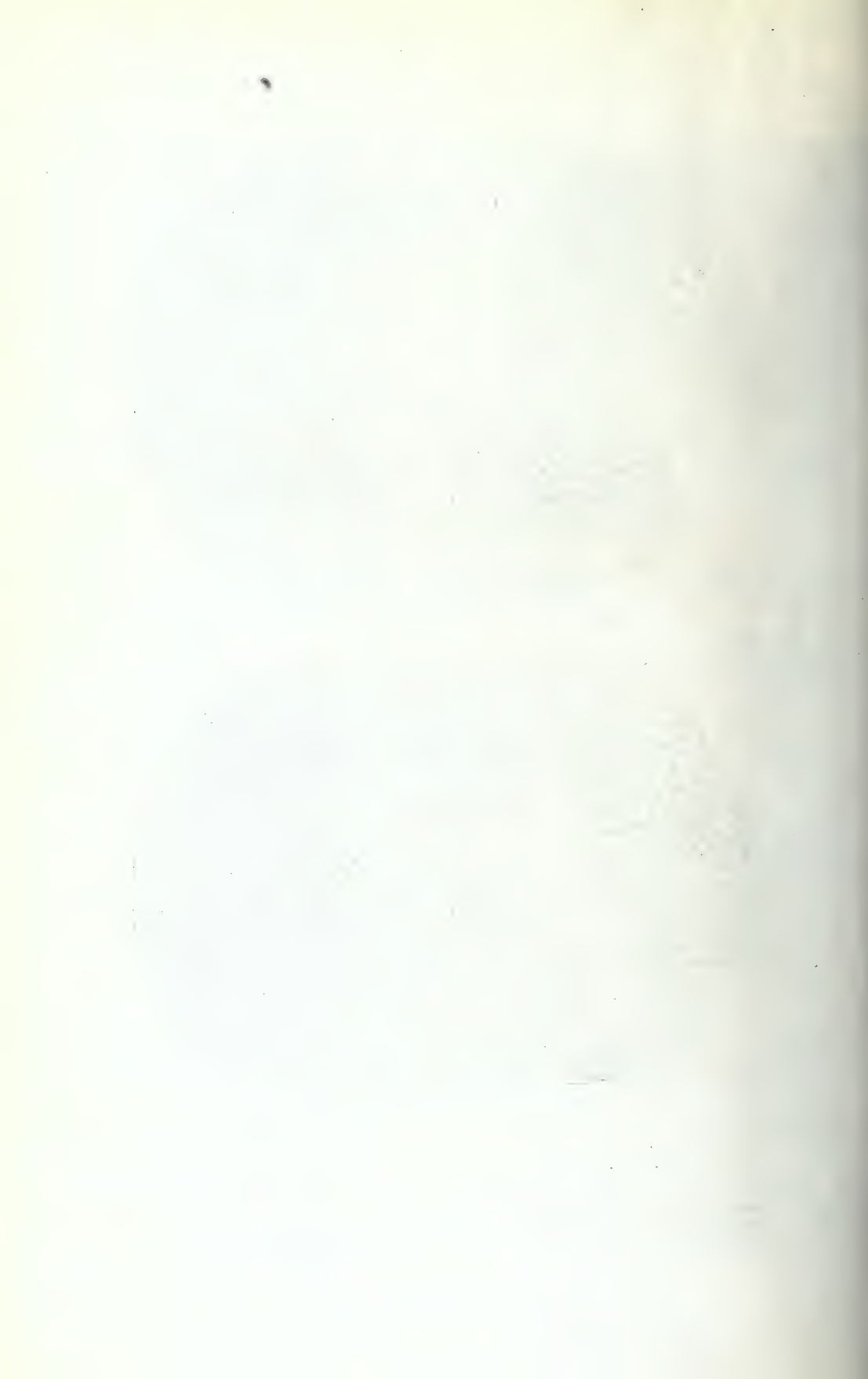
B

PLATE XVI

EXPLANATION OF PLATE

A. Pre-cirral canal, showing spines. ($\times 450$.)

B. Three nerve cells at base of oral suckers and others in pharynx. Note how freely pharynx is suspended in sinus of excretory system. ($\times 90$.)



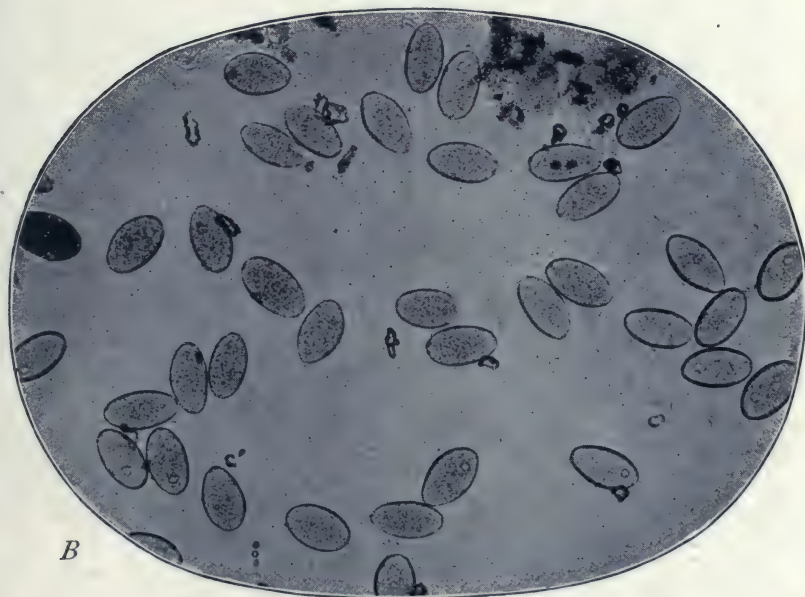
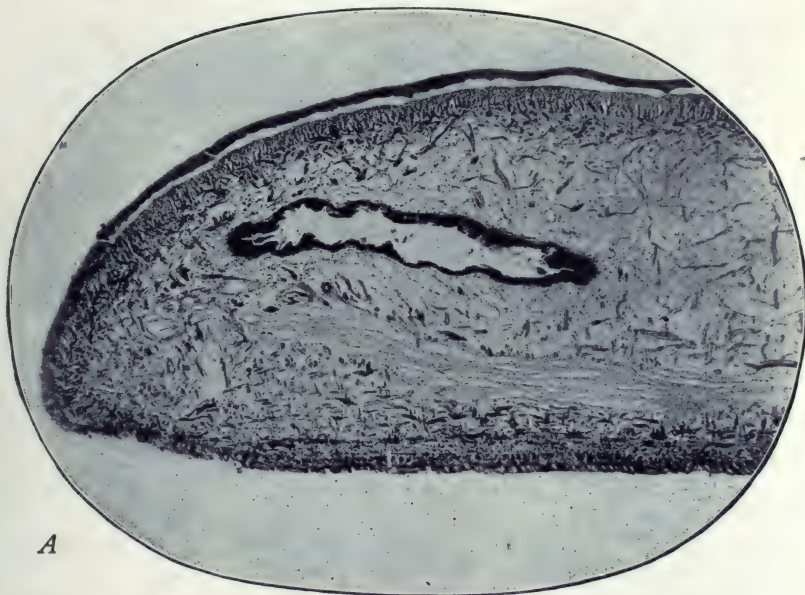
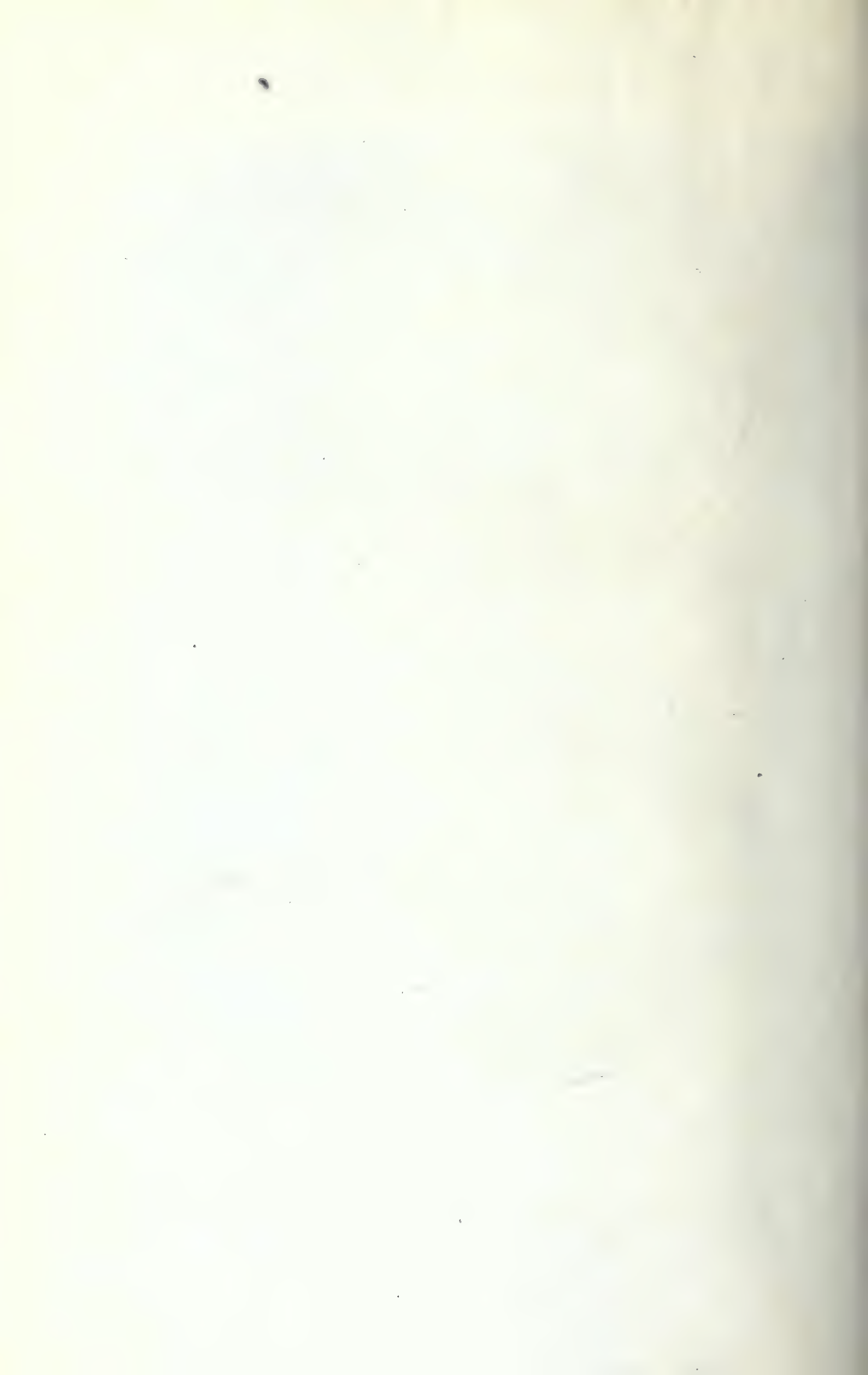


PLATE XVII

EXPLANATION OF PLATE

A. Sagittal section, showing nerve trunk at level of ventral sucker with a nerve cell near anterior extremity. Cecum at right. Note cuticula with spines on ventral surface, stripping off on dorsum. ($\times 45$.)

B. Group of eggs from feces. ($\times 68$.)



which it emerges as a tube 0.135 to 0.175 mm. in diameter. This portion of the tract, the esophagus proper, is very short (85 to 100 μ), as the transverse portion of the ceca lies practically in juxtaposition with the pharynx. The diameter of the ceca is not constant, but at the acetabulum approximates 230 μ and diminishes somewhat toward the tail. Up to the point of its bifurcation this canal is lined with an extension of the cuticula; beyond that point with tall columnar epithelium arranged in several (5-9) longitudinal ridges, which give to it a characteristic stellate appearance on cross section.

The excretory system begins immediately under the basement membrane of the cuticula as small spaces or canals which coalesce to form larger ones, and eventually empty into the excretory vesicle. In the anterior part of the body, three main trunks—a central and two lateral—gather up the smaller branches and unite at the lower border of the shell gland to form the excretory vesicle, which occupies the central portion of the fluke from this point to the extremity, with a diameter about one third of the thickness of the body at that part—approximately 500 or 600 μ . Throughout its extent it receives transverse and oblique tributaries, and from its caudal extremity a short, straight duct, 10 or 12 μ in diameter, leads to the external orifice on the posterior surface, about 300 μ from the tip (Pl. XIV, B). This excretory pore is provided with a sphincter in the dermo-muscular tube; the duct is lined throughout with cuticula, the vesicle with tall columnar epithelium, and the other branches of the system with a single layer of flattened cells.

Striking features of the anatomy of the fluke are the large sinuses surrounding the oral sucker, the ventral sucker, the pharynx, and to a less extent the shell gland and other organs, and communicating directly and freely with the main trunks of the excretory system. It seems reasonable to suppose that in addition to their excretory function these sinuses are analogous to the synovial or pleural sacs of mammals (Pl. XII, B, and Pl. XVI, B).

The Male Reproductive System.—The testes, two in number, consist of thick tubes, 0.6 or 0.7 mm. in diameter, and about one third the width of the fluke in length, which lie one behind the other ventral to the excretory vesicle, and with their branches occupy the greater part of the posterior half of the body. The branching is frequent and usually dichotomous, the finest divisions ranging from 116 to 200 μ or more in diameter. From each main trunk a vas deferens arises, and these pass forward over the surface of the shell gland on either side (Pl. XV, B), and converge to pierce the cirrus sac and empty into the posterior tip of the primary seminal vesicle. This point, the beginning of the cirrus sac, is usually easily made out in cleared specimens, and

lies ordinarily about midway between the shell gland and the tip of the acetabulum, but may be very much nearer the former.

The cirrus sac consists of muscular and fibrous tissue, and contains from behind forward the following structures: seminal vesicles, ejaculatory duct, cirrus and pre-cirral canal, the last three of these constituting the vas deferens. The first portion of the sac terminating at the tip of the acetabulum is more or less convoluted, and has a diameter of 500-700 μ . The second portion containing the vas deferens follows a straight course longitudinally, lying in apposition with the acetabulum in front, and with the metraterm to the left. Anteriorly, its walls blend with those of the genital atrium.

The seminal vesicles are two more or less convoluted tubes, lying side by side within the first portion of the cirrus sac. One of these vesicles, here termed the primary vesicle, extends caudad slightly farther than the other and receives the vasa efferentia. Its distal extremity empties into the lumen of the secondary vesicle (the so-called cecal appendage of the former descriptions) where it narrows to form the ejaculatory duct, but is directed caudad. Thus both vesicles are practically always full of sperm. Both vesicles are lined with a cylindrical epithelium.

The spermatozoön possesses the usual form—namely, a spindle-shaped body and a filamentous tail, with a total length of 6 or 7 μ . The ejaculatory duct, approximately the first half of the vas, is continuous with the seminal vesicle behind and the cirrus in front, and differs from the former only in being straight and smaller in diameter (140-200 μ). The cirrus forms about half of the remaining portion of the vas. The muscular fibers in its walls are notably developed and it is lined with very small and delicate spines, which hardly take the stain for chitin (Pl. XV, *B*). The distal extremity of the cirrus protrudes into the pre-cirral canal very much as the human cervix uteri extends into the vagina.

The pre-cirral canal which opens anteriorly into the base of the genital atrium, is closely set throughout with spines similar to the cuticular variety, but much smaller and more delicate, being 6.5x7 μ (Pl. XVI, *A*). When the cirrus is protruded this canal is entirely evaginated, forming then an outer coat enclosing the whole length of that organ except its tip. This doubtless will account for the statement by Stephens that the cirrus "is beset with very fine spines except at either extremity"; for the spines in the pre-cirral canal do not extend over the portion of the cirrus which projects into it (Pl. XV, *B*).

The genital atrium, into the bottom of which the pre-cirral canal and the metraterm empty side by side, is 250 to 300 μ deep, and when relaxed appears as a transverse slit some 400 to 500 μ wide. During

extrusion of the cirrus, however, that organ completely fills the lumen, and considerably distends it, giving it a cylindrical form. The atrium is lined with cuticula, but without spines.

The Female Reproductive System.—The vitelline glands are symmetrical structures, occupying the lateral portions of the fluke, and extend from the level of the acetabulum to the posterior extremity where they meet in the middle line. They lie superficially, immediately within the dermo-muscular tube, extending inward on both the ventral and dorsal surfaces for about one sixth of the width of the fluke, and thus enwrap to a certain extent the outlying parts of the testes, uterus, etc. Their acini are round or oval, and lined with large celled epithelium (18 to 20 μ), with prominent round nuclei (4 to 5 μ) and numerous refractile granules (2 to 2.5 μ). On each side the yolk substance is collected by numerous tubules into an anterior and posterior longitudinal tubule, which unite to form the transverse yolk duct, 100 μ in diameter. When near the shell gland the transverse ducts bend dorsalward to enter it in its dorsal and posterior portion, and expand at their junction within it to form the vitelline receptacle (Pl. XV, A), from which the common yolk duct, a small tubule, extends upwards and unites with the short oviduct to form the oötype.

The shell gland is an elastic body surrounded by a capsule of connective tissue, into which the parenchymal muscles are more or less blended. It is generally ovoid in shape with the long axis oblique to the midline of the body, the anterior end to which the ovary is attached being to the right of this line. In the smaller flukes it is situated but a millimeter or less anterior to the mid point of the body, but in the longest specimens lies at about the junction of the anterior and middle thirds. The cells of this gland are generally spindle-shaped (10x22 μ), but some are rounded (10 to 15 μ in diameter) with large, round, deeply staining nuclei (5 μ in diameter). These are gathered in a peripheral zone 100 μ or more in thickness, from which delicate processes converge toward the oötype.

The ovary consists of three stems—superior, middle and inferior—the outer ends of which are closely branched, while inwardly they soon merge into a common mass lying upon the upper right hand portion of the shell gland near the ventral surface. From this mass a small duct, the oviduct, sinks into the shell gland, takes a convoluted course to the posterior part of the gland where it turns toward the opposite side, gives off Laurer's canal, and immediately afterwards is joined by the common yolk duct to form the oötype. The cells of the ovary, or ova proper, stain a dark reddish brown with van Gieson, are generally nearly round (12 to 15 μ in diameter), with large, round, deeply staining nucleus (7 to 8 μ) and nucleolus (5 μ).

The oötype is a straight cylindrical tube, 100 to 135μ in diameter, extending transversely through the substance of the shell gland, and is continuous at either end with the oviduct and the uterus, respectively. It is lined with a layer of tall columnar cells lying upon a basement membrane, and in stained sections is surrounded to a depth of 50 to 60μ with deeply stained, radially arranged lines—the terminal portions of the processes of the cells of the shell gland—which give to it a characteristic caterpillar-like appearance.

Laurer's canal is a small duct given off from the oviduct just before that canal unites with the common yolk duct to form the oötype. It follows a convoluted course, especially in that part within and adjacent to the shell gland, and empties on the posterior surface in the mid line at a point a millimeter or so in a straight line from its origin. It is lined throughout with an extension of the cuticula, and is provided with a sphincter at its distal extremity. In general, its lumen is about 12μ , but frequent enlargements or sacculations of the tube occur, having twice or three times that diameter. There is no trace of any receptaculum seminis. Sperm was not found in any of the cases examined, but only a few cells from the ovary, and still more rarely yolk cells. Probably these were abnormally forced into the canal during the death spasm.

The uterus in the first part of its course is differentiated from the oötype by the absence of the caterpillar-like rays, and by its convoluted course. It leaves the shell gland near the upper left hand portion of its ventral surface, and arranged in loose coils occupies most of the anterior portion of the fluke as far as the acetabulum. In the larger flukes it is packed with eggs, and may attain a diameter of 500μ or more. The first portion of the uterus is lined with cylindrical epithelium—as is also the oviduct—but in the central portion this is reduced to a layer of thin, flattened cells with delicate processes projecting into the lumen like spines in appearance except that they do not stain. At the tip of the acetabulum the uterus merges into the metraterm, becoming reduced in size (about 150μ), the walls are thickened, muscular fibers especially being increased and the lining becomes identical with the outer cuticula, and is armed with stout spines measuring approximately $23 \times 28\mu$ (Pl. XV, B). These have been found embedded in the cuticle from the outer termination of the metraterm for somewhat over two thirds of its length and may possibly normally be found throughout its entire extent. Throughout its course the metraterm lies upon the acetabulum in juxtaposition with the vas and to its left.

The ovum is an ellipsoid, rather bluntly rounded at either pole, and measuring normally 130 to 140μ by 80 to 85μ . The shell is clear and

thin, the operculum so delicate as to be made out only with difficulty in many instances, and the contents consist of a large number of yolk cells with usually but a single germinal cell situated towards the operculum from the center. Considerable variations in size and shape are met with, though they are not common in eggs deposited in the normal manner, as is apparent from a study of Table 2, Graphs 4, 5, 6, and Pl. XVII, *B*; from these it also appears that the normal egg as found in feces is not so pointed toward the poles as it has been figured hitherto.

The Nervous System.—The nervous system may be divided into a peripheral and a visceral portion. The peripheral portion consists of a group of numerous ganglion cells clustered around the lower portion of the oral sucker (Pl. XVI, *B*), from which three nerves proceed on each side.

1. The ventral nerves pass rapidly forward toward the surface, and from the level of the acetabular orifice to their termination at the posterior extremity are found immediately below the dermo-muscular tube and slightly within the general course of the ceca (Pl. XVII, *A*). Anterior to the acetabulum they are united by three commissures, one at the base of the pharynx, and the others respectively above and below the transverse portion of the ceca. Throughout the remainder of their extent at intervals of from 300 to 700 μ they give off median and lateral branches which, with corresponding branches from the other nerves, are distributed to the entire periphery. Opposite the acetabulum the ventral nerves are 135 μ or more wide and somewhat less in thickness, and taper gradually toward the tail.

2. The dorsal nerves are superficial throughout their entire course, are united by a single commissure behind the cecum, and are somewhat smaller than the ventral nerves, but similar in their branchings and distribution.

3. The lateral nerves arise from the common origin and by a plexus of three or four branches from the ventral nerves on either side, and are distributed to the shoulders, terminating at about the level of the acetabulum.

Small bundles of fibers from the ventral nerves have been traced to the acetabulum and pharynx, and doubtless the two portions of the nervous system are intimately correlated, though this has not been demonstrated. But in general it is evident the portion of the system just described is concerned primarily with the innervation of the periphery, including the dermo-muscular tube.

4. The cells referred to as "ganglion cells" above, are elliptical cells about 38 μ long, and 30 μ in their short diameter. The nucleus is large and round, and the cytoplasm finely reticulated. In some

instances fibers have been seen to pass from the cell into the nerve bundle. As stated above, a large group of these cells is to be found around the base of the oral sucker, but single cells occur also at various points along the course of the nerves.

The visceral portion of the nervous system consists of round or oval cells similar to the ganglion cells of the peripheral portion, but without visible fibers leading from them. They occur in great numbers throughout the body, being most numerous in the powerfully developed muscles (oral sucker, pharynx, and ventral sucker), and in the cirrus and the metraterm; and to a less extent about the other viscera.

The foregoing detailed description may be summarized as follows: *Fasciolopsis buski* is a flat, elongated fluke, presenting great variation in size, but averaging about 30x12x2 mm. It is deep pink in color, and surface appears smooth except under microscope when ventral surface is seen to be beset thickly with spines. Oral sucker sub-terminal on ventral surface; ventral sucker powerful and near anterior extremity. Genital pore just anterior to ventral sucker. Vitellaria are racemose glands occupying lateral portions from ventral sucker to tail. Shell gland oval in mid line of body somewhat anterior to mid point. Uterus in loose coils occupies anterior portion of fluke, its terminal portion, the metraterm, being spined and emptying into the common genital atrium beside the pre-cirral canal. Ovary, closely branched, attached to shell gland on the right. Testes, two in number, closely branched, and lying one behind the other, occupy most of the body posterior to the shell gland. Cirrus and pre-cirral canal lie parallel with metraterm, and are armed with fine spines. Excretory vesicle large, with many transverse and oblique branches, and empties on dorsal surface, near posterior extremity.

CONCLUSIONS

1. Infestation with *Fasciolopsis buski* is to be regarded as a serious disease; and where local conditions favor it becomes of considerable importance from the standpoint of public health.

2. The most notable symptoms are general weakness, diarrhea, anemia, and edema. The rapid accumulation of fluid in the body may be accompanied by a pronounced suppression of urine without evidence of renal involvement.

3. Contrary to certain authorities, fever is not noted, except in complicated cases.

4. The parasite shows great variation in morphology, but without such gradation in variation as to justify including the forms now described as *F. rathousi* and *F. goddardi* in the species *F. buski*. On

account of the close similarity of *F. fülleborni*, it would appear desirable to subject this species also to further investigation.

5. Ventral cuticular spines are characteristic of *F. buski*, and probably of the entire genus.

6. Cirrus and metraterm are spined in *F. buski*, and therefore Raillet's description of the family Fasciolidae, if it is still to include Fasciolopsis, needs to be revised accordingly.

ACKNOWLEDGMENTS

Grateful acknowledgement should be made here of my indebtedness to the China Medical Board of the Rockefeller Foundation for the grant of a fellowship without which this investigation would have been impossible, and to Prof. E. E. Tyzzer of the Department of Comparative Pathology of Harvard University under whose direction it has been carried on, and whose cordial interest in the work has been manifested in many helpful ways. It is a pleasure to express my indebtedness also to my colleague, Dr. C. H. Barlow, of Shaohing, China, for aid in securing some of the material; to Prof. H. B. Ward of the University of Illinois for helpful suggestions, and to Prof. F. B. Mallory of Harvard University, and his able assistant, Miss Lillian M. Leavitt for the fine series of microphotographs which accompany this article.

NOTES ON SOUTH AFRICAN CERCARIAE *

ERNEST CARROLL FAUST

During the last few years Capt. F. G. Cawston of the South African Medical Corps has published several articles on South African cercariae. But, as Cort (1919:488) has so aptly remarked, "his descriptions and figures of this [cercaria of *Schistosoma haematobium*] and other forked-tailed cercariae which he has described are so entirely inadequate that it seems to me that his entire work needs verification by more competent observers." The writer has made a careful analysis of slides and alcoholics of species which Cawston has sent to Professor Henry B. Ward and presents the data in this paper.

Cercaria gladii Cawston 1918 (Fig. 1)

This furcocercous cercaria, found in *Isidora schakoi* at Potchefstroom, Transvaal, is of striking interest because of its conspicuous tail, the forks of which are prolonged into long, sword-like processes. The larva measures 0.25 mm. in length by 0.073 mm. in width. The main portion of the tail is 0.29 mm. long, while the furci have a maximum length of 0.38 mm. The body is distinctly glandular and the tail is conspicuously muscular. The integument is entirely covered with minute spines. The oral sucker has a transverse diameter of 33μ . The acetabulum lies about two-thirds the body distance from the anterior end. It is only 26μ in diameter. The oral sucker opens into a short undivided gut without evidence of any pharynx. Cawston's Figure 3 (1918:69) is a very inadequate and inaccurate diagram of this cercaria. Heavy mucin ducts empty thru the ventral margin of the oral sucker, rather than farther anteriorly, as in many furcocercariae. The openings are tipped with hollow piercing spines. Each of the two groups of ducts can be traced back to three large mucin glands, with relatively small nuclei and a network of granules in the cytoplasm. A mass of many germ cells is found near the posterior margin of the body. The central nervous system is unique in its position at the inner end of the gut. From it extend caudad two main ventral nerve trunks and delicate dorsals. No cyst granules have been found in the cercaria. It is quite unlikely that the larva encysts.

* Contributions from the Zoological Laboratory of the University of Illinois, No. 133.

Cawston has made no mention of the sporocyst of this species. Material which the writer has examined shows it to be muscular at the anterior end.

Cercaria secobii Cawston 1915 spec. inq.

Because of the poor mounts which have been available for study little can be added to Cawston's confusing description of *Cercaria secobii*. The data taken as a whole show clearly that this cercaria, first secured from *Physopsis africana* from the Umsindusi River, Pietermaritzburg, Natal, after the host had been subjected to an invasion of miracidia of *Schistosoma haematobium*, are not the cercariae of human bilharziosis. Cawston (1915: 257) states that "the evidence is not absolutely conclusive that these cercariae were present as a result of their exposure to infection of miracidia; but, in view of the recent work done by Leiper in Egypt, the inference is allowable." Such an inference is entirely without justification either from the experimental or the anatomical data.

Our actual knowledge of this species may be summed up in the statement that it is a furcocercous cercaria with a body about 150 by 24 μ , an undivided tail trunk 200 μ long and furci equally long, developing within a sporocyst, without pigment eye-spots and probably without pharynx. It is further distinguished from the cercaria of *Schistosoma haematobium* by a somewhat smaller, narrower body and by longer tail furci. The mucin gland ducts are probably the "divided gut" of Cawston's description. The really diagnostic features of number and character of mucin glands and ducts are altogether wanting.

Cercaria secobii is apparently confined to the coastal region where *Physopsis africana* abounds.

Cercaria parvoculata Cawston 1919 (Fig. 2)

This species, found in *Physopsis africana* at Durban, Natal, has a body measurement of 120 x 50 μ , a tail trunk 220 to 250 μ long, and furci about 100 μ long. Its oral sucker is pyriform. It possesses a small, weak acetabulum, and three pairs of acidophilic mucin glands with small nuclei. A clump of germ cells is found just posterior to the mucin glands. The pair of minute eye spots is found midway between the mucin glands and the base of the oral sucker.

A most remarkable fact in connection with *C. parvoculata* is the development of the cercariae in simple sacculate rediae, which are distinguished from the usual parthenitae of the group (sporocysts) by the presence of true pharynges and rhabdocoel ceca.

Cercaria of Schistosoma haematobium (Figs, 3, 4, 5)

These cercariae have been described and figured by Leiper for Egypt and by Cawston and others for Natal. For the former territory *Bullinus* spp. have been found to serve as hosts for the invading miracidia, while in Natal *Physopsis africana* is the mollusk involved. The life history of this species has been thoroly established by the brilliant work of Leiper. Unfortunately, this writer has almost entirely overlooked the anatomical features of the larvae. Only the grosser features have been included in his diagrams (Leiper, 1915). These are so general as to serve no purpose in the identification of the related larval schistosome species. While the shape and relative proportions of the body, tail trunk and furci are important, items of greater diagnostic value for furcocercous cercariae are the exact type of the digestive system, including number and type of mucin glands and ducts, the number of germ-gland cells, the nervous system, and the number and relation of flame cells and excretory tubules.

Specimens of this cercaria from Natal which the writer has studied have permitted an analysis of the digestive system, including the mucin glands and ducts, and the germ cells, together with the external body features. The body of the cercaria averages about 0.24 mm. in length by 0.1 mm. in width, while the tail trunk measures 0.2 mm. long by 47μ in diameter at the base. The small blunt furci are about half as long as the tail trunk. The large oral sucker gives the larva a decidedly robust appearance. The acetabulum is small and weak. Body and tail are covered with minute spines which are heavier and longer at the anterior end of the body. The oral sucker leads into a digestive tract without any evidence of pharynx. An esophagus runs backward into ceca which extend about three-fifths the distance caudad. Paired groups of mucin-gland ducts empty their slimy contents at the outer margin of the oral sucker. Each duct opens thru a hollow piercing spine which caps the duct (Fig. 5). Each group can be traced back to three mucin glands in the region of the acetabulum. These cells have loosely scattered granules in the cytoplasm and large nuclei. No other mucin glands have been found. Several germ cells have been found in the region of the body posterior to the acetabulum. The number is considerably in excess of the number of testes in the adult worm.

In specimens of the cercariae of *Schistosoma mansoni* from Caracas, Venezuela, which the writer has been enabled to examine thru the courtesy of Dr. Juan Iturbe, the mucin glands consist of only two pairs of cells of the granular type, but, in addition, four pairs of a non-granular type, somewhat smaller and surrounding the granular cells. The ducts are decidedly heavier than in the South African species. They open thru six spinose protuberances which cap the ducts.

To clear up Iturbe's work on this species it is necessary to state that the figure in Iturbe's paper (1917) is not a photomicrograph, but rather a diagram more nearly corresponding to the cercaria of *S. japonicum* than to the actual cercaria of *S. mansoni* which Iturbe found in the vicinity of Caracas.

Recently Cort (1919) has made a study of the cercaria of *S. japonicum*, which is altogether the most thorough anatomical analysis yet made of a human schistosome larva. The number of flame cells on each side of the body is four, rather than five as Miyairi and Suzuki found; likewise, the number of mucin gland cells is five for each side of the body, whereas the Japanese investigators considered the number to be three. As he says, the difficulty in differentiating the three human schistosome cercariae "is undoubtedly due to the limitations of our knowledge than to a lack of specific differences."

TABLE FOR DIAGNOSIS OF SPECIES OF HUMAN SCHISTOSOME CERCARIAE

	<i>S. haematobium</i>	<i>S. mansoni</i>	<i>S. japonicum</i>
Size:			
Body	240 x 100 μ	140 x 60 μ	100-210 x 66 μ
Tail trunk	200 x 47 μ	200 x 27 μ	150 x 20 μ
Furci	80-100 μ long	50 μ long	75 μ long
Oral sucker	60 μ in transection x 64 μ in length	30-34 μ in transection x 30-34 μ in length	33 μ in transection x 54 μ in length
Mucin glands	3 pairs with large nuclei and granular acidophilic cytoplasm	2 pairs with large nuclei and granular acidophilic cytoplasm; 4 pairs with small nuclei and basophilic slime contents	5 pairs with large nuclei and granular acidophilic cytoplasm
Mucin ducts	Moderately thick	Very thick	Very thick
Duct openings	At anterior end of oral sucker; capped by 3 pairs of hollow piercing spines	At anterior end of oral sucker; capped by 6 pairs of hollow, piercing spines	At anterior end of oral sucker; capped by 5 pairs of hollow, piercing spines
Germ cells	Several large cells posterior to acetabulum	Many cells at posterior end of body	Clustered mass of cells just behind acetabulum
Parthenita	Sporocyst	Sporocyst	Sporocyst

Neither in Cort's description nor his figures is the exact relationship of the piercing spines to the openings of the mucin gland ducts clear. While he may be correct in assuming that these glands "of the fork-tailed cercariae appear to be homologous to the stylet

glands of the xiphidio-cercariae which open at the base of the stylet or piercing organ, and to which the function of dissolving tissue in connection with the penetration of the cercaria into its host has been ascribed by certain authors," he is not exact in his statement that "instead of a single stylet as in the xiphidio-cercariae, the schistosome cercariae have a number of spines around the openings of the cephalic gland which perform the same function as the stylet in penetration." For each one of these piercing spines, hollowed in the center, caps the opening of a mucin gland duct, and it is thru this hollowed spine that the secretion of the gland is poured forth (Figs. 4, 5). This has been clearly demonstrated not only in the cercariae of *S. haematobium* and *S. mansoni* and in *Cercaria gladii*, but also in many undescribed schistosomes which the writer has studied, as well as the larval echinostome, *C. acanthostoma* Faust. The numbers of piercing spines for each mucin gland group is five in the cercaria of *S. japonicum*, altho Cort has figured five on one side of his drawing, and only four on the other side. This exact relation of a piercing spine to the opening of each duct has been borne out in every case which the writer has examined. Thus for *Cercaria gladii* and the cercaria of *S. haematobium* there are paired groups of three hollowed spines, while in the cercaria of *S. mansoni* there are six piercing spines to each group.

Cercaria catenata Cawston 1917 (Fig. 6)

Cawston's basis of diagnosis of this species which was found in *Planorbis pfefferi*, *Lymnaea natalensis* and *Physopsis africana* at Durban, Natal, was the "chain of blackish granules" which "lay on each side of the divided alimentary canal." Even with this apparent distinction Cawston has labelled certain specimens of *Cercaria catenata* "tadpole cercariae." A more thoro study shows the presence of a collar of spines just behind the oral sucker. This fact, among others of critical value, places the species among the larval Echinostomidae.

Contrary to Cawston's designation of the species as a large form (1917: 131), the writer has found it to be small, especially small for the group to which it belongs. The body measures 0.26 mm. in length by 0.13 mm. in width. The tail is 0.4 mm. long and only 36μ in diameter at the base. The oral sucker is 24μ in section, and the acetabulum 43μ . The latter is sunk in a deep circular depression which has a diameter about twice that of the acetabulum.

The oral sucker leads thru a very short prepharynx into a small pharynx. From the pharynx the esophagus, almost capillary in structure, runs posteriad to the anterior margin of the acetabular depression, where it gives rise to ceca of very small diameter. These end just behind the posterior margin of the depression.

The main tubules of the excretory system are characteristically echinostome. From the sides of the transversely compressed bladder two lateral collecting tubules run forward just outside of the ceca. They continue almost as far as the plane of the pharynx where they flex outward and backward. A single median tubule runs backward from the bladder thru the tail. It forks at the very end of that organ so that two outlets are formed. Cawston's "chain of blackish granules" refers to the granules in the excretory system.

The germ cells in the cercaria consist of a group of units in the median line posterior to the caudal margin of the acetabular depression and another mass on the anterior margin of the depression. These are connected by a chain of cells. The body of *Cercaria catenata* is crowded with cystogenous cells. The cyst granules in the cells are long narrow bodies closely packed together side by side. Judging from this provision, encystment must occur rapidly in this species.

Cawston has described the redia of *C. catenata* as a robust animal with four walking legs, a pointed posterior end, and a large gut filled with orange-colored material.

Cercaria constricta nov. spec. (Fig. 7)

Liver tissue of *Physopsis africana* which Cawston sent from Natal has been found to contain rediae and cercariae of a new larval echinostome for which the name *Cercaria constricta* is proposed. The body of the larva measures 0.19 mm. in length by 0.1 mm. in width, while the tail averages 0.28 mm. in length by about 40μ in section at the base. The entire body and tail are covered with very sharp spines directed posteriad. In the region lateral to the pharynx there is a deep constriction of the body which sets off the head from the trunk. A collar of sharp spines, larger than those covering the body as a whole, runs in a single series along the anterior margin of the constriction. It is complete except for a small gap on the ventral side just below the pharynx.

The acetabulum has a width measurement of 53μ and a length diameter of about 35μ . The oral sucker has an average diameter of about 35μ . The digestive tract consists of a short prepharynx, a small muscular pharynx, a long esophagus reaching to the anterior margin of the acetabulum, and furci which extend to the subcaudal region of the body. Mucin glands have been seen, but their arrangement in the body has not been exactly determined.

The excretory bladder is quadrangular in shape and quite muscular. From the median posterior margin a single caudal tubule runs half the distance distad, where it splits into two tubules. These tubules open laterad in the subdistal region of the tail. An anterior collecting

tubule from each of the anterior angles of the bladder runs forward to the plane of the pharynx, where it reflexes and continues backward. Further than that the writer has not been able to make out its course. The tubules contain no excretory granules.

Conspicuous germ-gland cells have been observed just anterior to the acetabulum.

The redia is an elongate sac without feet, with a minute obovate pharynx, a simple gut and a deep constriction in the region of the neck. A birthpore has not been observed. The redia is entirely covered with spines.

It will be noted that *Cercaria constricta* differs from *C. catenata* in several important points. Prominent among these are the spinosity of the cercaria and redia of *C. constricta* and lack of such integumentary differentiation in *C. catenata*; difference in type of excretory tubule in tail, and absence in *C. constricta* of the circular depression found around the acetabulum of *C. catenata*. The lack of excretory granules in *C. constricta* is probably of specific value, but this point needs checking with living material. A further distinction between the two species is found in the presence of four feet in the redia of *C. catenata*, while the redia of *C. constricta* lacks feet entirely.

That *Cercaria arcuata*, described by Cawston (1918a:95) from the Transvaal, is still a different species of larval echinostome is shown from the fact that the species has two redial feet, and an anterior collecting system which empties thru a single median stem into the bladder.

Cercaria arcuata Cawston 1918 (Fig. 8)

The original description of this species was made by Cawston on the material from *Isidora* sp. from the Schoonpoort at Klerksdorp; the material which the writer has had an opportunity to examine is from *Lymnaea natalensis* from Natal. As in other echinostome larvae which Cawston has described, he has referred to this species as a "leptocercous cercaria" (1918a:95).

The body of *Cercaria arcuata* is 0.15 mm. long by 0.1 mm. wide in the region of the acetabulum. The tail is about one and one-half the length of the body. The body is entirely covered with spines which are most conspicuous at the anterior end. A distinct collar prominence has been seen on the worm, but no collar spines have been made out with certainty. The anterior end of the body is capable of considerable extension. The oral sucker measures 37μ in diameter, and the acetabulum has a diameter of 33μ .

The redia is a long sac with a pair of pointed feet about in the middle of the body. At the anterior end of the body pharynx, collar prominence and birth pore can be readily seen. The rhabdocoel gut

may be small or large, depending on the age of the redia and the amount of food ingested.

Contrary to Cawston's description, the writer has been able to make out a prominent pharynx just dorsal to the oral sucker. It leads into a long esophagus. The furci arise just anterior to the acetabulum and continue nearly to the posterior limit of the body.

The excretory bladder is an elongate median organ extending nearly to the posterior margin of the acetabulum. Here it forks in horse-shoe fashion to form two dilated collecting tubules which run forward to the region of the pharynx. It is filled with a few very large excretory granules, the "chain of cystogenous vesicles" of Cawston. The finer portions of the collecting tubules have not been made out. The collecting tubule in the tail forks soon after it enters that organ. The two branches continue distad and open to the exterior in the subdistal region of the organ.

The body is filled with an enormous amount of cystogenous granules, which obscure all the finer structure of the worm. On encystment these granules are extruded from the body, forming a covering around the decaudated worm.

Cercaria cawstoni nov. spec. (Fig. 9)

This is one of the "tadpole cercariae" which Cawston has recorded for *Physopsis africana* and *Lymnaea natalensis* from Natal. A comparison of the figure accompanying this description with Cawston's several figures of this type shows how entirely inadequate and misleading his description is.

The larva is ovate-oblong, with a slight protrusion at the oral end and an impocketing at the posterior end into which the tail fits. The body measures 0.38 mm. in length by 0.21 mm. in width. The tail measures 0.31 mm. in length by 43μ diameter at the base. The body is entirely covered with minute spines, but the tail is aspinose. At each side of the caudal pocket is a studded cluster of long, heavy spines, which are imbedded in a thickened region of the integument (Fig. 9, 9 b). The oral sucker has a diameter of 43μ , while the acetabulum measures about 60μ . Imbedded in the dorsal wall of the oral sucker is a stylet, 27μ long. This organ is of the simple quill type, but is unique in having a median longitudinal reinforcement in addition to the usual transverse thickening (Fig. 9 a).

From the oral sucker the digestive track is traced thru a short prepharynx to a minute pharynx, 16μ in diameter. From this region a short esophagus leads to the ceca which extend nearly to the posterior end of the body. Emptying at the sides of the stylet are paired groups of mucin gland ducts, each group arising from four mucin

cells. These cells have large vacuolated nuclei and a homogeneous chromophilic cytoplasm. The excretory bladder is a transversely constricted organ into which empties a common collecting tubule. This tubule forks just posterior to the acetabulum. A single mass of germ cells has been found posterior to the acetabulum.

The body of *Cercaria cawstoni* is filled with cystogenous granules. When the cercaria is freed from the ruptured sporocyst it drops its tail and encysts.

Cercaria frondosa Cawston 1918 (Fig. 10)

Cercaria frondosa is a sturdy amphistome larva measuring 0.4 mm. in length by 0.31 mm. in width, with a tail 0.43 mm. long by 57μ in section at the base. The oral sucker has an average diameter of 66μ , while the acetabulum, 95μ in diameter, is situated at the posterior margin of the body and not on the ventral side as Cawston has figured it (1918:69). The parthenita is a large, heavily walled muscular redia, varying in size, but always possessing a prominent pharynx, a long, slender gut and a birth pore. The worm was found in *Isidora schakoi* at Potchefstroom, Transvaal.

Internally, the oral sucker leads into a cavity with distinct pharyngeal pockets, which probably argues for its place among the Diplodiscinae. The short esophagus is not surrounded by a postpharyngeal sphincter. It opens into a rather inconspicuous pair of ceca which run posteriad to the region of the acetabulum. The bladder is small, but collecting tubules which empty into it from the side, are enormously dilated by excretory granules. The two main tubules can be traced forward to a region under the pigment areas immediately behind the eye-spots. A single tube, running thru the middle of the tail, forks near the proximal end of that organ to open thru small pores to the exterior. The germ cells of the cercaria consist of a clump of elements in the median line just behind the anterior limit of the lateral excretory tubules.

Two eye-spots are present. They are of a bee-hive shape, with the pigment cup opening anterolaterad. The optic cell is conspicuous in the young cercaria long before the pigment granules accumulate around it. Spreading out from the eyes in frondose arrangement are pigment elements which show under high magnification a grouping into flaky masses that at times extend over the entire dorsal surface of the animal.

Large rhabditiform cystogenous granules pack the parenchyma cells of the cercaria. Upon the maturing of the cercaria either within the liver gland of the snail or after wandering out of the host, the cystogenous granules are thrown out to form a heavy cyst membrane

With encystment the tail is dropped and the larva passively awaits transmission to the subsequent host. Cawston has called this larva a leptocercous distome.

Cercaria fulvoculata Cawston 1919 (Fig. 11)

Cawston called this species leptocercous, but it must be placed among the larval monostomes. It is ovate in outline, with slight auricular prominences on each side near the anterior end. The body is 0.4 mm. long and half as wide. The tail is heavy and about 0.6 mm. long. It is provided with six paired groups of long falciform cells surrounding the caudal excretory canal. The body has a small but prominent oral sucker and a pair of aspinose caudal pockets. The cercaria is binocular, with flecks of pigment surrounding the eye spots and at times extending backward along the nerve tracts. The cercaria was taken from *Lymnaea natalensis* at Durban.

A large bladder lies mesad near the posterior end of the body, with lateral conduits opening into it from the sides. These ducts connect with one another just posterior to the eyes. In front of the bladder is an ovarian cell mass, and ventral to the cornua of the bladder are small testicular germ masses. Ducts from these glands run forward in parallel courses to the region of the genital pore, which is situated behind the plane of the eye spots.

The redia is a simple sacculate structure with medium-sized pharynx and long slim gut. There are no feet. A birth pore has not been seen.

DISCUSSION

A survey of the data above shows the inadequacy of Cawston's descriptions. In a private communication he has stated that the illustrations are most unsatisfactory, but are very similar to those of Drs. Leiper and Atkinson in the *British Medical Journal*. This is no less unfortunate. It is true that Leiper's descriptions and figures will not serve to separate species of larval schistosomes, because all details of structure are omitted. Leiper's statement (1915: 39) that the systematic position of a bifid-tailed cercaria can only be effectively established in the first instance by experimental infection of a susceptible host and the subsequent examination of the adult resulting therefrom is misleading and entirely out of accord with the facts. Specificity of structure is as characteristic of the larval fluke as of the adult, and failure to find specific differences between cercariae is due to inadequate observation.

These difficulties are generally felt, and Leiper (1918: 168) states that there is no evidence that the various forms so loosely and repeatedly termed "*Bilharzia cercariae*" in Cawston's numerous papers

EXPLANATION OF PLATE

Fig. 1.—*Cercaria gladii*, ventral view, showing digestive, nervous and reproductive systems. $\times 170$.

Fig. 2.—*Cercaria parvoculata*, ventral view of body only, showing digestive and germ-cell glands. $\times 330$.

Fig. 3.—*Cercaria* of *Schistosoma haematobium*, ventral view, showing digestive glands and germ cells. $\times 170$.

Figs. 4 and 5.—Anterior tip of cercaria of *Schistosoma mansoni*. 4, mucin ducts and openings at anterior margin of sucker; $\times 330$. 5, tips of ducts, showing hollow spine capping each duct. $\times 990$.

Fig. 6.—*Cercaria catenata*, ventral view, showing digestive and excretory systems and germ cells. $\times 170$.

Fig. 7.—*Cercaria constricta*, ventral view, showing digestive and excretory systems. $\times 170$.

Fig. 8.—*Cercaria arcuata*, ventral view, showing digestive and excretory systems. $\times 170$.

Fig. 9.—*Cercaria cawstoni*, showing digestive and excretory systems and single mass of germ cells; *a*, stylet, enlarged; *b*, lateral view of cluster of caudal pocket spines, enlarged. $\times 170$.

Fig. 10.—*Cercaria frondosa*, ventral view, showing pigmentation around eye-spots, digestive and excretory systems and single mass of germ cells. $\times 170$.

Fig. 11.—*Cercaria fulvoculata*, ventral view, showing pigmentation around eye-spots, excretory and reproductive systems. $\times 170$.

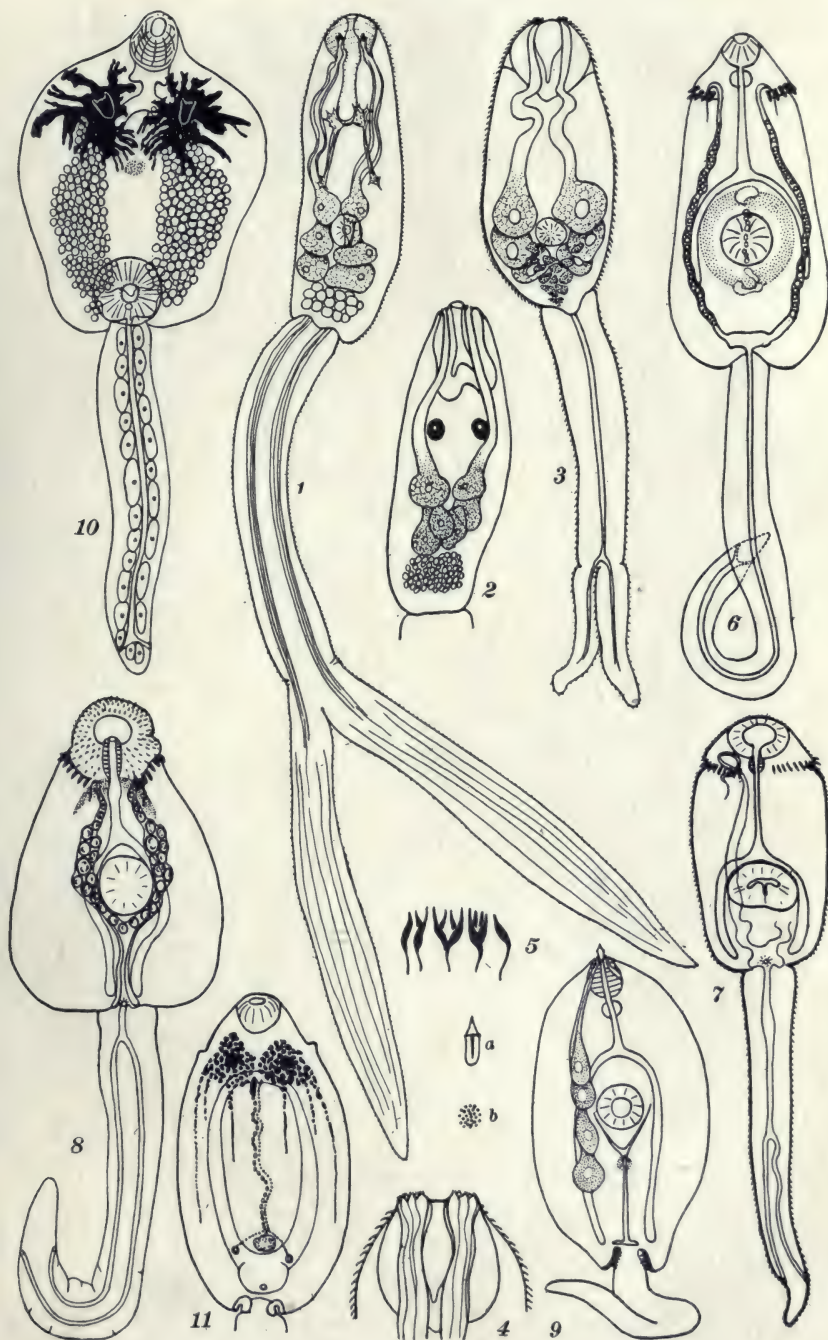
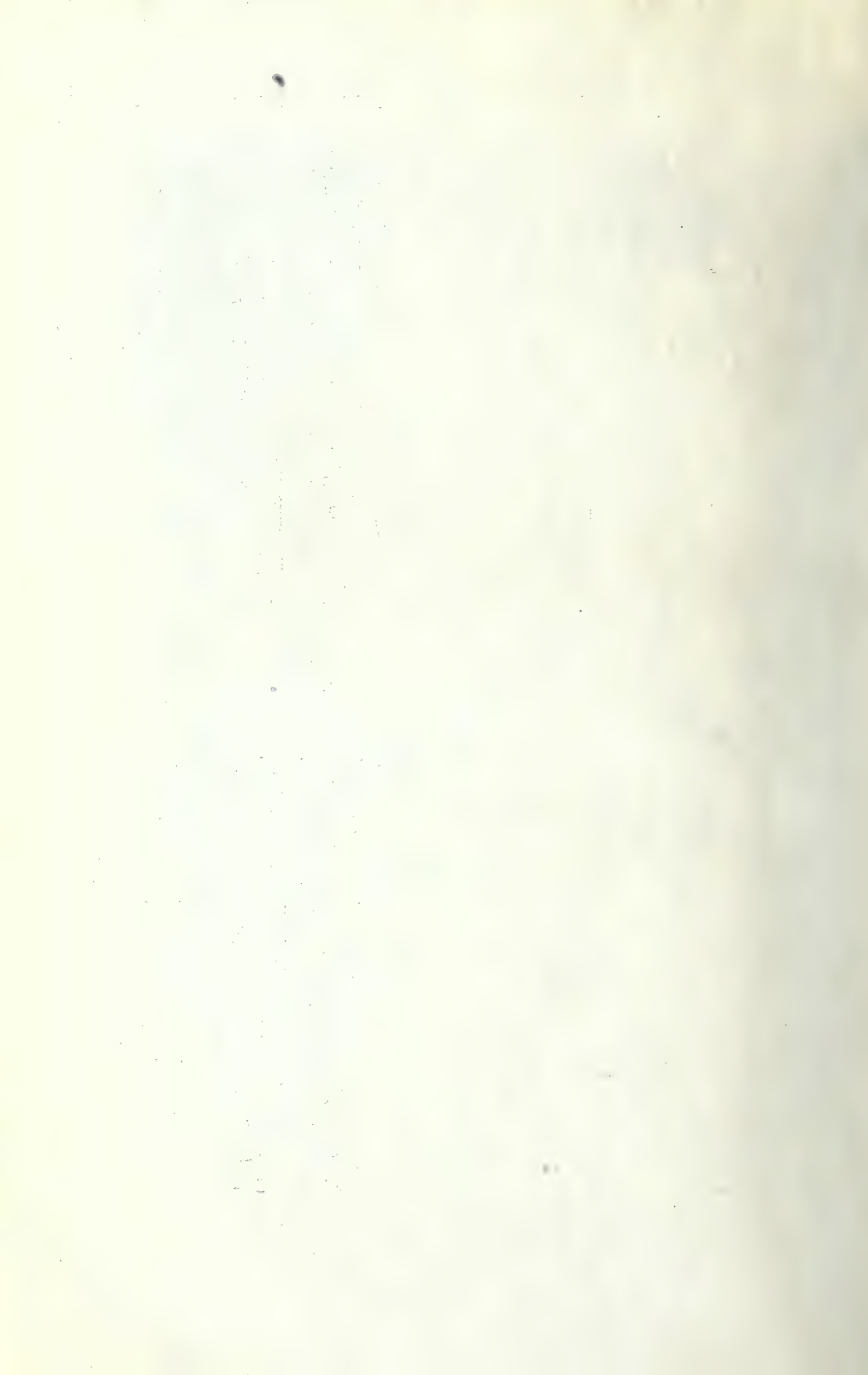


PLATE XVIII



are actually such. In a review of one of Cawston's papers (1916: 348) new figures are substituted for those in the original article (1915: 258). A comparison fails to show a single common likeness between any two of the figures, altho they are labelled "bilharzia cercariae." In another paper Cawston (1916a: 201) has labelled at least three distinct species (Cercaria of *S. haematobium*, *C. oculata* and *C. catenata*) as "human forms of Cercariae," altho the latter forms cannot possibly be considered human forms.

SUMMARY

1. Ten species of cercariae from South Africa, including two new, are described.

2. The cercariae of *Schistosoma haematobium*, *S. mansoni* and *S. japonicum* are easily distinguished on the basis of number and type of mucin glands and ducts, and their outlets. Differences in arrangement of the germ cells may also be used in this diagnosis.

3. Diagnosis of a larval trematode requires exact data on the size and shape, on the integument, on the excretory system, on the digestive system and on the germ cells.

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TETRADONEMA PLICANS NOV. GEN. ET SPEC.,

representing a new family, TETRADONEMATIDAE as now

found parasitic

IN LARVAE OF THE MIDGE-INSECT *SCIARA COPROPHILA* LINTNER

N. A. COBB

United States Department of Agriculture

HABITAT AND OCCURRENCE

Number, Location and Maturity of the Parasites.—Of this parasitic nema, both male and female are found in about equal numbers in the body cavity of the larvae of the midge insect identified by Professor H. B. Hungerford as *Sciara coprophila* Lintner, often as many as six to twelve of the parasites being found in a single larva. Adult males, about one-sixth as long as their mates, are usually found coiled about females, and both males and females are more or less entangled with the malpighian and tracheal vessels of the host, so as often to be rather difficult of extraction. These facts give rise to the specific name *plicans*. The generic name was suggested by the highly interesting four-celled organ, the tetrad, located in the anterior part of the nema. The parasites occupy a very considerable part of the body cavity of the host. In the material examined some of the nemas of each sex were surrounded by cast-off cuticula, indicating that they moult at least once after they enter the host. The fully matured females contain thousands of eggs having somewhat the form of an immature mushroom cap (Fig. 1). The shells of the eggs are smooth and of medium thickness, and contain embryos in various stages of development. The most advanced embryos seen appeared to be taking on a serpentine form and to be coiled once to twice in the egg. When, in the course of dissection of preserved material, the largest females become broken, their eggs escape in large numbers into the surrounding fluid. Preserved eggs escaping in this way are about 33μ in greatest diameter.

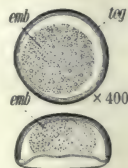


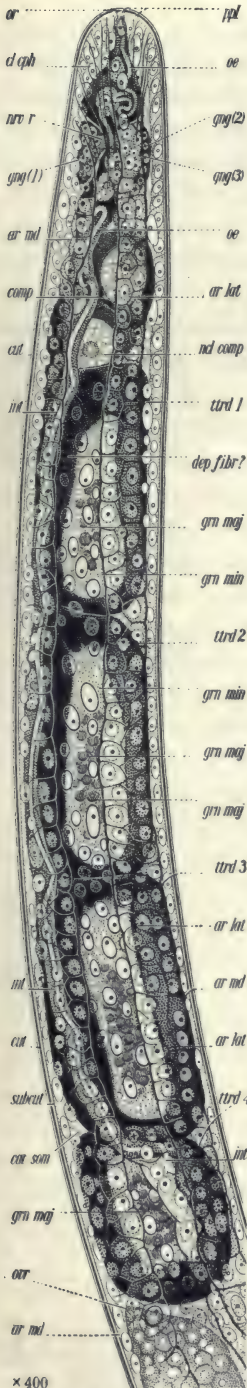
Fig. 1.—Two views of eggs from the uterus of *T. plicans*. *emb*, embryo; *teg*, shell of the egg.

SIZE AND FORM OF TETRADONEMA PLICANS,

Dimensions. The Formula.—Below are measurements of male and

0.0	0.9	2.2(?)	⁵⁰ / ₆₀	⁴⁰ / ₄₀	08.5	5 mm.	0.0	5.7	9.3(?)	⁷¹ / ₇₁	M	87.	
0.0	0.8	0.8			2.4	0.9	0.0	3.1	3.6		5.2	4.2	0.8 mm.

female. The figures are averages derived from four males and two females, prepared by fixing the host in picro-aceto-sublimate and pre-



serving in 70 per cent. alcohol, and finally mounting in glycerin jelly. The tissues of the head of *T. plicans* appear susceptible of contraction, and it seems not unlikely, from the appearance of the preserved specimens here described, that the heads had become fixed in a somewhat retracted attitude, and, if so, the measurements must be interpreted accordingly; see Figure 2, in which the tortuous esophagus may indicate a retracted condition of the head. Similar retractions may occur in the heads of other nemas, e. g., *Oxyuris vermicularis*, a fact that has led some writers into a *non sequitur*, and consequent disparagement of the decimal formula. Such changeableness of form has no more to do with the method of expressing the measure-

Fig. 2.—Head end of a mature female of *Tetradonema plicans*, nearly lateral view. The entire tetrad is shown—occupying the greater part of the illustration. *ar lat*, lateral field; *ar med*, median field; *cav som*, somatic cavity; *cl cph*, nucleus of cephalic cell; *comp*, cells referred to as companion cells of the tetrad; *cut*, cuticula; *dep fibr?*, semifibrous deposit between the nuclear membrane and the cell wall of one of the members of the tetrad; *gng* 1, 2 and 3, groups of nerve cells connected with the nerve-ring; *grn maj*, the major granules of the tetrad elements; *grn min*, minor granules of the tetrad elements; *int*, alimentary canal; *ncl comp*, nucleus of cell referred to as companion cell of the tetrad; *nrv r*, nerve ring; *oe*, esophagus; *or*, mouth; *ovr*, ovary; *ppl*, cephalic papillae; *subcut*, subcuticula; *ttrd* 1, 2, 3 and 4, the four elements of the tetrad; compare with Figure 3.

ments than the market value of wheat has to do with the currency in which it is expressed. The same may be said of other variabilities of form, as well as uncertainties of observation. The limits of variability may be expressed in the formula in the usual way, by using the two limiting figures. Uncertainty as to measurement may be expressed by an interrogation mark, as in the above formulae, where the location of the beginning of the intestine is indicated as questionable. Notwithstanding the uncertainty, the queried figures, $\frac{9.3}{3.6}$ and $\frac{2.2}{0.8}$, are as useful as ever in indicating the contour of the body.

STRUCTURE OF THE NEMA

Cephalic Organs and Body-Wall.—The general appearance of the tissues of the head and of the neck of *Tetradonema* is reminiscent

of that of some members of the Mermithidae, e. g., *Mermis nigrescens*; in both forms numerous nuclei of cells in the head are of relatively large size, and the filiform extensions by which the cells are connected with one another and with the cuticle are more or less similar in appearance in the two forms. In immature *Tetradonemas* the characteristic tetrad, a group of four cells, or "cysts," to be described later, is not yet fully developed; in young individuals its cells are distinguishable from other cells, for instance the larger spermatocytes lying in the testis near at hand, mainly by the fact that they are larger, and that their spherical nuclei have a more pronounced fine granulation. The head of *T. plicans* is hemispherical-conoid, then sometimes almost imperceptibly truncated at the narrow mouth opening. Occasionally one sees immediately around the lipless mouth what appear to be about six very obscure forward pointing innervations, and at other points toward the margin of the head one occasionally sees what appear to be innervations that may represent papillae (Fig. 4, *ppl*). There are no amphids or eye-spots or other pigmented tissues of any kind. The coarser, non-equidistant, transverse striations of the cuticula are indicated by fine transverse lines that break joints opposite the lateral fields, the average distance between them being about equal to one fifteenth the width of the body. Between the transverse refractive lines just mentioned there are others uniformly spaced, the true striae of the cuticula; these are barely visible with lenses of the highest power used under favorable circumstances, and are not further resolvable (Fig. 8, *str*). No definite wings have been seen. The lateral fields as seen near the middle of a male undergoing its final moult and viewed in profile appear to be about one-half to three-fifths as wide as the body. Beginning very narrow near the head, the lateral fields widen out rapidly, so that at the anterior extremity of the tetrad they are fully half as wide as the corresponding portion of the body. The fields contain large and relatively conspicuous, somewhat ellipsoidal nuclei that are more or less granular, and contain distinct nucleoli. A double row of these nuclei is the main feature of each lateral field. The median fields are of similar size and contain nuclei of a similar character (Figs. 2 and 3, *ar med*, *ar dsl*, *ar vnt*).

Longitudinal striae. Muscles.—On each side of each lateral field in the region of the neck are longitudinal striations, which either exist in the cuticula or are due to the existence of attachments of

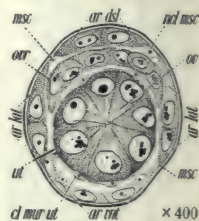


Fig. 3.—Cross section near the vulva of *T. plicans*. *Ar dsl*, *lat*, and *vnt*, dorsal lateral and ventral fields; *cl mur ut*, cell of uterine wall; *msc*, submedian muscular field; *ov*, ovum in uterus; *ovr*, ovary; *ut*, uterus; *ncl msc*, nucleus of muscle cell.

somatic cells, presumably muscle cells. These narrow and weak longitudinal muscular fields are submedian in position, and each is represented by about four striae. The transparent tissues of the neck permit of seeing these submedian, narrow, longitudinally striated elements more clearly than they can be seen elsewhere, but they have been followed far backward, and no doubt exist throughout the length of the body (*msc*, Fig. 3). There exist in the neck, as elsewhere, not only lateral fields, but also median fields. In one of these, the ventral, the row of nuclei is more definite and the nuclei are somewhat larger than in the other.

DEGENERATE ALIMENTARY CANAL

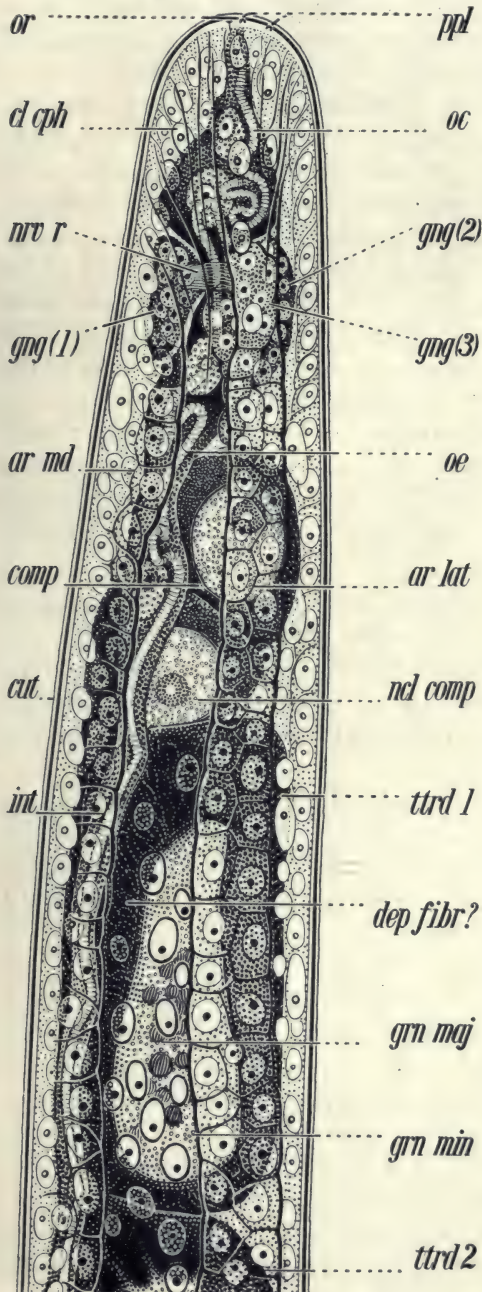
The digestive system of the adults of both sexes is more or less degenerate or vestigial, as, it appears, is often the case with nemas inhabiting the body cavities of insects, e. g., *Allantonema*, *Sphaerularia*, *Tylenchus*, etc. It may not be an unreasonable supposition that to some extent the food of *T. plicans* is absorbed through its cuticula, since fully adequate means for imbibing it through the mouth seem to be lacking.

Alimentary Canal, Male.—There is no pharynx. From the mouth opening backward the esophageal tube is very narrow, but may present an almost imperceptible swelling just in front of the nerve-ring. This latter lies about half way to the tetrad and is nearly transverse. There are filamentous processes passing from it to the body wall, presumably nerves. Near the front of the tetrad may be dimly seen what appears to be the junction of the esophagus with the intestine. Just in front of this point the esophagus is very slightly swollen; the posterior portion of the esophagus therefore appears somewhat narrowly clavate in form, and is about one-fourth as wide as the corresponding part of the neck. In some specimens, however, this swelling was sought in vain. The succeeding part of the alimentary canal (the intestine, or the posterior part of the esophagus, as the case may be) is at first about as wide as the part of the esophagus just described, but soon diminishes in size and becomes a rather insignificant looking strand of indefinite tissue, containing a faint lumen. The alimentary canal soon passes to the ventral part of the body, and is so inconspicuous and deteriorated that one is usually unable to follow it further. In one case, at the nerve ring, which was about half way back to the first member of the tetrad, the esophagus was not more than one-fifth as wide as the corresponding part of the neck, and thence backward it diminished in size and was very difficult to follow. In the male the esophageal lumen does not seem to lead through a granular plasma as is the case in the female illustrated in figures 2 and 4. In immature males a rectum is present, and, joining it, a portion of the

intestine can be seen, extending forward a distance somewhat greater than the length of the tail, and at its widest part becoming half as wide as the body. At a point as far in front of the anus as the terminus is behind it, the intestine is smaller, and farther forward still it is difficult to follow and seems very rudimentary. This condition of things exists in those males whose testes are filled with spermatocytes about one-fifth as wide as the body; that is to say, somewhat immature males.

Alimentary Canal, Female.—An examination of the females shows that, just as in the male, the alimentary canal is much deteriorated, but the details are somewhat different (Fig. 2, *int*). Immediately behind the mouth opening the lumen of the canal becomes tubular and more or less tortuous. The diameter of the more or less corrugated lumen is about equal to the thickness of the cuticula. Surrounding the median canal is a granular tissue or "plasma" in which large nuclei are to be seen here and there. From the mouth backward this granular tissue expands so that at a distance from the anterior extremity one and one-half times as great as the diameter of the head it may become about half as wide as the corresponding part of the neck. Immediately behind this point, however, there is a constriction, and in the midst of this constriction the nerve-ring is found (Fig. 2, *nrv r*). Immediately behind the nerve-ring the intestinal canal, or esophagus, as I believe we may still term it at this point, gradually widens until it becomes one-third as wide as the body; it then again diminishes in size so that anterior to the two large cells in front of the tetrad it is only about one-fifth as wide as the corresponding portion of the body. This portion of the body seems to be what would be called the base of the neck, and if so, this constriction, for such it seems to be, corresponds with the beginning of the intestine. Behind this constriction the alimentary canal again widens and soon becomes about one-fourth as wide as the body, and then once more begins to decrease. However, even as far back as the last member of the tetrad it still appears to have a tubular lumen. At this point the tubular lumen suddenly ceases, suggesting the possibility that the esophagus really extends farther back than indicated above (Fig. 2, *int*). No trace of the alimentary canal was seen farther back than the middle of the body. As the alimentary canal, for a certain distance at least, has a distinct lumen, and there is a distinct mouth opening, small though it be, it seems likely that the intestine is still capable of taking in liquid nutriment. It is possible that the large nuclei associated with the anterior part of the alimentary canal, of which half a dozen may be counted in the esophageal portion just described, may have something to do with assimilation. It has been assumed that certain nemas parasitic in the

body cavities of insects absorb their nutriment through the cuticula, and there is good cause to suppose that in some instances this may be



so. However, when the alimentary canal of the parasite contains a distinct lumen, and the mouth opening is still a distinct feature of the head, it is a fair assumption that the intestine may still function to a certain extent, especially if there are associated with it structures whose office may easily be supposed to be accessory to digestion or absorption.

Fig. 4.—Head end of a mature female of *Tetradonema plicans*; nearly lateral view. Compare with Figure 2, *ar lat*, lateral field; *ar med*, median field; *cl cph*, nucleus of cephalic cell; *comp*, cells referred to as companion cells of the tetrad; *cut*, cuticula; *dep fibr?*, semifibrous deposit between the nuclear membrane and the cell wall of the front member of the tetrad; *gng* 1, 2 and 3, groups of nerve cells connected with the nerve ring; *grn maj*, major granules of the tetrad; *grn min*, minor granules of the tetrad; *int*, alimentary canal; *ncl comp*, nuclei of one of the cells referred to as companion cells of the tetrad; *nrv r*, nerve ring; *oe*, esophagus; *or*, mouth; *ppl*, cephalic papillae; *ttrd*, one of the elements of the tetrad.

THE TETRAD

Structure of the Tetrad.—A very striking feature of the anatomy is the occurrence, toward the head end, of four large unicellular organs, arranged tandem, and occupying in this region the greater portion of the body cavity (Fig. 2, *ttrd*, 1, 2, 3 and 4). It is this quartet of bodies, the tetrad, that gives rise to the generic name *Tetradonema*.

That each of the four elements of the tetrad is unicellular is shown by the fact that in the younger nemas, where the tetrad is smaller, it is

quite clear that each of its elements is a single cell containing a large spherical nucleus having a distinct nucleolus. In the full grown female the tetrad is much more strongly developed, and the entire space between the cell wall and the nuclear membrane of each element is completely filled with a semi-fibrous, semi-granular deposit (Figs. 2 and 4, *dep fibr?*). Under these circumstances the form of the cells is no longer spherical, but more or less cylindrical, owing to very great increase in size and consequent pressure from surrounding organs. In the males, also, the tetrad cells may become so large that the nuclei are no longer spherical, though this seems less commonly the case than in the females. The nuclei of the tetrad may become half as wide as the body, the deposit surrounding them then becoming thicker and more opaque, and seeming to be more "fibrous" the older it becomes. The tetrad in the male may be of such a size as to be twice as long as the distance between its anterior extremity and the mouth opening. Accompanying the tetrad, and in front of it, are to be seen two smaller more or less spheroidal cells that seem to be larger in the female than in the male (Figs. 2 and 4, *comp*). Occasionally each of this pair of cells is so large as to suggest that they are "companions" of the cells comprising the tetrad.

Function of the Tetrad.—Such a striking organ as the tetrad of *Tetradonema* cannot but give rise to the question, "What is it for?" I have been unable in the examination of the small amount of preserved material available to make out the histological connections of the tetrad, but the following facts are clear as a result of the examination made.

1. The organ is found in both sexes in the same form and consists of four unicellular, apparently equivalent, components, which develop from comparatively normal cells lying near the base of the neck, the usual location of the renette, and seem to grow with the age of the organism rather than with its size.

2. As the nema ages these elements not only increase in size, but also change in structure, one of the principal changes being a bulky, apparently semi-fibrous deposit just inside the cell wall. Meanwhile the nucleolus becomes a locus rather than a cell organ, and finally there are deposited in the very much enlarged nucleus, relatively large, spherical, more or less structureless granules (Figs. 2 and 4, *grn maj*). Sometimes these major granules have a distinctly refractive element. As these larger granules appear only in the larger tetrads, the possibility is suggested that they are a degeneration phenomenon, but I am more inclined to regard them as excretory in nature, along with the semi-fibrous matter outside the nucleus. The nucleus maintains its membrane to near the close of the history of the tetrad.

3. It is noticeable that no trace of the usual excretory pore and renette has been seen in any of the specimens.

Is it possible that the cells of the tetrad are storehouses for excreta? The food of this nema may be predigested, but the catabolism must give rise to waste matter. Now there is no true anus; nor has any excretory pore been seen. To this statement of the entire absence of the main channels through which excreta are usually voided, may be added the suggestion that should the parasite pour its excreta into the body fluid of the host, presumably the effect on the host would be injurious, and this in turn would be inimical to the parasite. If such a thing were possible, it would seem advantageous to the nema

under the circumstances to store up within its body the wastes of its own metabolism, the excreta due to its growth and reproduction. The data thus far disclosed leave it possible to suppose the tetrad to have some such function. On the other hand, no such organ is known in any other parasitic nema, though of course it is conceivable that organs having the function here imagined but more obscure or of smaller size, might hitherto have escaped notice. It is desirable that the tetrad be studied in living specimens, and be submitted to chemical tests.

Sexual Organs of the Male.—The single spiculum is median in position, and without accessories. There is no bursa and there are no ventral supplementary organs, and no distinct caudal papillae. Oblique copulatory muscles are to be seen for some distance in front of the anus. The ejaculatory duct is about one-half as wide as the body. In somewhat immature males the main portion of the two testes already nearly fill the body cavity, and contain many thousands of spermatocytes, whose average diameter is about one-fifth to one-sixth that of the body. Immediately behind the tetrad a flexure is to be seen in the anterior testis. At this point the testis suddenly diminishes considerably in size and extends thence backward and ends; this blind end of the anterior testis seems to lie toward the middle of the nema, and is nearly one third as wide as the corresponding portion of the body. Toward the posterior end of the nema, as far in front of the anus as the terminus is behind it, there is a definite

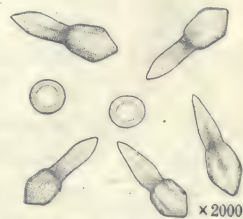
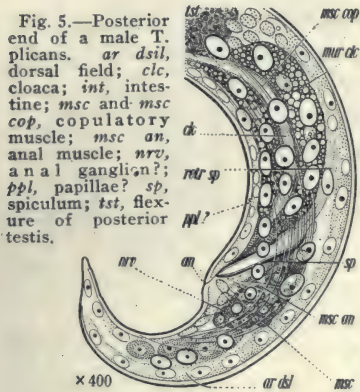


Fig. 6.—Sketches of the sperm cells of *Tetradonema plicans*. From various points of view.

broad contour line indicating the presence of a flexure of a similar character in the posterior testis. The two testes meet near the middle of the body where their junction with the vas deferens is more or less plainly visible. The fully mature spermatozoa found in the *vas deferens* of the male and in the uterus of the female are somewhat asymmetrically elongated-fusiform bodies about one-sixth as long as the body is wide, and about one-third as wide as long. Packed together with them in the proximal portion of each testis are more or less finely granular, spheroidal, apparently non-nucleated bodies of various sizes, the largest being one-fourth as wide as the body of the nema, and the smaller having not more than half this diameter. As these spheroidal bodies are most numerous and most apparent near the proximal ends of the testes, it is assumed that in the ripening of the sperm these bodies are formed. The ripened spermatozoa occur in thousands and are reminiscent of those of *Mononchus*, *Dorylaimus* and *Anticoma*. In the uteri of the female they have more or less the contour of tadpoles (Fig. 6). The sperm cells appear to be produced in groups, possibly in groups of four. It was found difficult to make an accurate count, so closely were they packed in the testes, but the number of individuals in a group is certainly small.



Fig. 8.—Tail end of female. *ar dsl*, *lat* and *vnt*, the dorsal, lateral and ventral fields; *cl ar lat*, *vnt* and *dsl*, cells of the fields; *ovr*, posterior flexure of the ovary; *str*, striae.

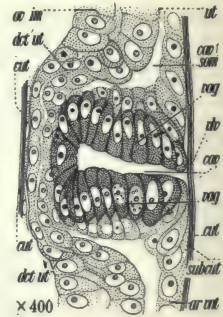


Fig. 7.—Optical section vulvar region of *T. pliocans*. *ar vnt*, ventral field; *cav som*, somatic cavity; *cav*, cavity of the vagina; *cut*, cuticula; *ds ut*, uterine duct; *ov in*, oocyte; *subcut*, subcuticula; *ut*, uterus; *vag*, wall of the vagina; *vlt*, location of the vulva.

Sexual Organs of the Female.—From the rather inconspicuous vulva, the very strongly developed vagina passes inward at right angles to the ventral surface three-fourths the distance across the body. It is composed of a bulky mass of cellular tissue three-fourths as wide as the nema, and is one of the main features of the middle of the body (Fig. 6). Its distinct cavity appears rather narrow, and is lined with a layer consisting of scores of closely packed, relatively large, elongated cells. This more or less columnar “epithelial” lining is the main feature of the developing vagina. Outside it, however, there is a layer of smaller cells placed somewhat irregularly. From the proximal part of the vagina, toward the dorsal side of the body, two comparatively narrow tubes lead in opposite directions to the two uteri, one in front, the other behind. Each uterus is about three to four times as long as the body is wide, and in young females appears to be about half as wide as the body. Where the uterus joins

the ovary there is a faint constriction, and the contour of the organs is here so definite as to make it evident that the ovaries are reflexed (although I usually found it impossible to clearly identify the reflexed portion throughout its length), for a longitudinal optical section at this portion of the body discloses ovarian tissue other than that comprised in the portion of the ovarian tube that joins the uterus. This ovarian tissue extends to near the vagina, from which it is evident that the reflexed portions of the ovaries reach back to near the vulva. These details were made out from the study of nearly mature but unfertilized females. The flexure in the anterior ovary is at the back end of the tetrad; the flexure in the posterior ovary is only a short distance from the end of the tail (Figs. 2 and 8 *ovr*).

Tail.—The female has no anus, nor is there any vestige of such an opening. However, its former location may be estimated from consideration of the position of the anus on the male, whose tail end has a similar form. With this estimate in mind it becomes evident that the tail of the female begins to taper some distance in front of the position of the theoretical anus. The tail of the female is rather like that of the male in form, being at first conoid, but ending in a subcylindroid or somewhat convex-conoid terminus, about one fifteenth as wide as the body, and about two to three times as long as wide, and having a more or less acute tip. Considering the tail to comprise that portion of the posterior end extending from the flexure of the posterior ovary to the terminus, this final narrow portion of the tail occupies about one-third of its length. On this portion of the tail the transverse striae of the cuticle can be seen more plainly than on almost any other part of the body. There are, of course, no caudal glands.

New Family of Nemas.—I consider *Tetradonema*, of which the type species is *Tetradonema plicans*, to be the type genus of a new family, the Tetradonematidae.

TETRADONEMATIDAE fam. nov.

TETRADONEMA gen. nov.

Small naked insect-parasites with minute males; cuticula wingless, minutely transversely striated; head rounded, tail acute; mouth minute, lipless, oral papillae minute, anus none; esophagus simple, with lumen, intestine vestigial; male and female gonads double, symmetrically reflexed; vulva central, uterine eggs numerous, asymmetrical, containing embryos; spiculum single without accessories, supplements and bursa none.

BIOLOGICAL NOTES ON *TETRADONEMA PLICANS*,
COBB, A NEMATODE PARASITE OF *SCIARA*
COPROPHILA LINTNER

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University of Kansas

INTRODUCTION

In January, 1915, while studying the life history of the Mycetophilid fly *Sciara coprophila* Lint., which is often found breeding in potted plants, the writer found one batch of maggots parasitized by a peculiar nematode. The unusual appearance of this worm led to a study of its life history. Several photographs and figures were made at the time as a matter of interest, but aside from recording the effectiveness of this parasite in the destruction of *Sciara* maggots,* nothing was done until the beginning of 1918, when the nema under discussion was sent to Dr. N. A. Cobb for determination.

DISTRIBUTION

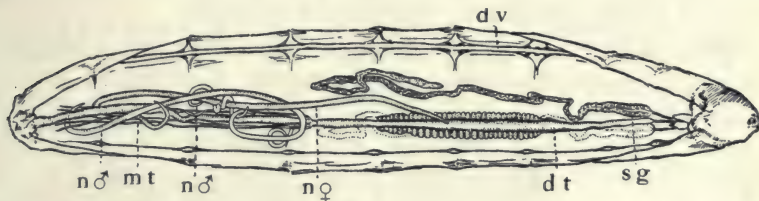
In an endeavor to determine the range of distribution, the number of specific hosts and the percentage of infestation in nature, a careful search has been made of every *Sciara* fly and maggot obtainable. These were collected from fields of alfalfa and of wheat, from meadow grasses, from beneath the leaves in the woods, conservatories, and from green houses, but aside from the material cited at the beginning of this paper, none was ever found parasitized by this nematode. Grub worms and angle worms living in similar situations have been found free also. Experiments to infect these last have failed, although I have seen small angle worms swallow the eggs on several occasions. These angle worms were, in every case, isolated, and kept under observation. They did not become infected.

A study of the one lot of infected *Sciaras* and those artificially infected in the experimental work shows that the gravid female parasite may be found in larvae, pupae and adult flies. From two to twenty parasites of both sexes may be found in a single host; on the average, ten worms came from each host, and the number of males ran a little greater than of females.

* In the Journal of Economic Entomology for December, 1916, the writer mentioned this nematode as an enemy of *Sciara* maggots and figured the gravid female nematode.

APPEARANCE OF AFFECTED HOST

When one compares a normal maggot with an infected one, a marked contrast is noted (Figs. 1 and 2). In the former, the maggot appears white, due to the large definite fat bodies present. There are present also segmentally arranged fat masses about the spiracles. The head capsule is shiny black, and, as shown, not conspicuously smaller than the diameter of the anterior part of the maggot. On the other hand, the parasitized maggot gives no evidence of either the long fat masses or the segmentally arranged fat bodies. The head capsule is very likely to be small, indicating the failure of the maggot to make its normal moults. The nematodes within appear white by reflected light (Fig. 2). An examination of such a maggot shows plainly the pulsations of the dorsal vessel, with its muscular wings and pericardial cells, are most plainly seen. In one maggot the female parasite was disrupted in such a way as to release her eggs into the body cavity of the maggot. These eggs were seen buffeted about within the transparent skin of the maggot by the movement of its organs. The dorsal



Sciara maggot dissected, *d v*, dorsal vessel; *d t*, digestive tract; *s g*, salivary gland; *m t*, malpighian tubules; *n*, nematode.

vessel was burdened with the continuous coursing of nematode eggs from the rear to the front. Several times the eggs temporarily clogged the dorsal vessel near its anterior outlet, only to break away under the pressure like a released log jam and go racing on with the circulatory fluids.

EFFECT UPON THE HOST

Larva.—The young maggot of 2 mm. may have the body teeming with newly hatched nemas, which are visible under the microscope, without showing much deviation from normal, but very shortly the long fat bodies begin to decrease in size. In the normal maggot these fat bodies are long masses that cling to the salivary glands and surround the malpighian tubules, and the lateral masses, segmentally arranged close to the muscles of the body, one on either side, also a smaller mass beneath each nerve ganglion. These finally disappear altogether, leaving the body very clear. The digestive tract, with its appendages, the heart and the nervous system, can all be seen plainly.

A little later the digestive tract seems subnormal in diameter, but still functional. If the maggot is slit longitudinally, fixed, stained, and the muscular portion and integument spread and mounted upon a slide, the muscular system will present a conspicuous appearance, all the nuclei staining well. The maggot at this stage retains muscular activity, but later remains extended and incapable of movement. A stained mount of such a maggot would show the muscular system in the state of collapse, the heart action being about the only evidence of life. In spite of the general disintegration, the imaginal disks are present, as also the lateral segmentally arranged oenocyte clumps. Maggots, when heavily parasitized, die and disintegrate, leaving within or upon the earth only a mass of several thousand nematode eggs.

Pupa.—Sometimes in light or late infection, the maggot endeavors to pupate; it spins out threads and sheets of silk, then contracts, as is usual before transforming. This marks the end of some; others succeed in casting off the larval skin. Many such pupae are little else than mere shells, the body cavity being filled with the egg-burdened nematodes. In no case could I find traces of reproductive organs, either free or attached in infested pupae of either sex.

Adult.—Adults emerging from the jars containing infected maggots were in most cases parasitized. They could fly about, but were lacking in reproductive organs. The few uninfected flies contained normal reproductive organs, but there was little difference in the appearance, especially of the females, for the abdomens of parasitized flies were swollen with the mature female nematodes. The direct economic aspect of the case of parasitism reported herein lies in the fact that the maggots which feed upon plant roots are destroyed or rendered less active, and the fact that those fortunate enough to transform to the adult fly stage are rendered incapable of perpetuating their kind.

LIFE HISTORY OF TETRADONEMA PLICANS COBB

This nematode has but the one host, which may be larvae, pupa or adult of the *Sciara* fly. Possibly it may live also in other species than the *Sciara coprophila* in which it was found.

There is considerable dimorphism in the mature nematode. The females are large, reaching a length of 5 mm. or more, while the males are less than 1 mm. long. The striking characteristic of the female is shown in the mature egg laden form (Fig. 4). The swollen portion is caused by the storage of several thousand eggs beneath the cuticula, which serves as a retaining capsule. The notch on the ventral side of the worm marks the position of the genital opening. The relative sizes of the two sexes some time after mating is illustrated in Figure 3. Here the female has begun to lay her eggs as shown by the spindle-

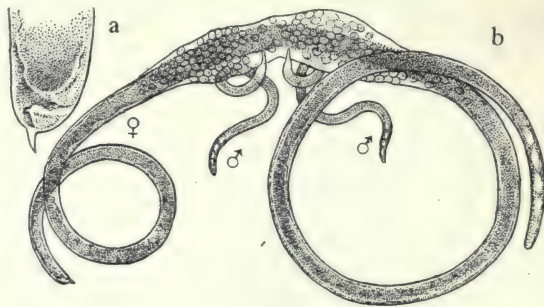
shaped capsule; this continues to enlarge until the female has the appearance shown before but, barring accident, the eggs are not discharged until the death of the worm.

Method of Infection.—The nematode eggs very often have been induced to hatch in the presence of moisture. All efforts to observe the young larvae enter the maggot have failed. When first discovered in the host, they appear identical with those just escaped from the egg and have been detected first in the caudal end of the maggot in the region between the body wall and the digestive tract. The older maggots are much less susceptible to infestation than the younger ones. When young maggots not more than 3 mm. long were placed in earth containing the nematode eggs, they would, in the course of a day or two, be found to contain sometimes as many as twenty-one young parasites. The parasite probably gains access to its host through the alimentary canal. Even newly hatched maggots are of sufficient size to consume solid objects larger than the egg of the nematode. The larger maggots habitually swallow bits of earth and solid pieces of organic matter many times the size of the eggs. As a matter of fact, I have found the eggs of this nematode in the digestive tract of small *Sciara* larvae, and believe that the young nemas hatching from eggs that have been swallowed, bore through the wall of the digestive tract into the body cavity of the maggot.

Percentage of Infection.—As stated elsewhere in this paper, only one lot of infected material has been found. In this case every maggot was infected. Material from this lot was used to make artificial infections.

Development and Behavior of the Host.—The life cycle of this nematode has been followed from its earliest observed stage to the mating of the sexes and the formation of the egg capsules about the females, all of which takes place within the body of the maggot, and is clearly visible due to the transparency of infested larvae. The young nematodes as they are found in the maggot are of two kinds and of the same size as those that have hatched from eggs in a drop of water. This precludes any possibility of an alternate host. The method of studying the life cycle was to examine carefully young maggots taken from noninfested stock and then place them in small stenders with earth containing nematode eggs. Examinations were made several times daily. The eggs mixed with the debris swallowed by the maggots were not to be noticed in the living maggots, but when the latter were killed and the digestive tracts removed and cleared in cedar oil, as many as a dozen nematode eggs were found in the mid gut of one maggot. In the living maggots the first newly hatched

worms were to be noted in the body cavity at the posterior end of the body. Here they would often get into the way of the blood stream entering the dorsal vessel, and be buffeted back and forth. The slenderer worms were usually coiled at both ends, while the shorter plumper forms remained outstretched or slightly curved. From this time on the growth of the worms is rapid and usually timed, so that their eggs are produced before the maggot is ready to pupate. In cases of slight or late infection, the nematode cycle may not be completed until the pupal stage has been reached or even the adult fly produced. In adult flies have been found gravid females, heavily egg laden and also small nematodes equaling in size those of a few days of age. These I take it have been arrested in their development by the growth and maturity of older worms. Indeed even in a given maggot isolated for study the range of development of the various worms has been so great that I have found it difficult to tabulate the stages.



a, Caudal end of mature female. *b*, Female with two males attached.

On the average, very young parasites have been found in maggots from 1.5 mm. to 4 mm. long. Mating begins when the female nematodes are less than 1 mm. long, a size they may attain in from five to ten days. The remaining growth of the female, which attains some 5 to 7 mm. in length, and of the egg capsule involves the next two weeks.

Mating.—As the worms approach sexual maturity, marked activity is noted which may precede mating by a day or two. They begin to coil and uncoil about each other in an energetic manner. The males are the more active, coiling about the body of the female with the caudal end almost indiscriminately, until finally the genital opening is grasped, and the male rests with caudal end tightly clasped about the female, and the anterior end directed away at about a right angle. The spicules hold the males so firmly in position that they are often difficult to dislodge with dissecting needles. As many as three or four males may become attached to one female, and remain until the female completes

her egg capsule and dies. The males that remain unmated finally are relegated to the caudal end of the female, where they become granular in appearance and sluggish in action, finally dying. The males remaining attached to the female also show this granular appearance.

Oviposition.—The eggs of this nematode are retained beneath the thin cuticula of the female. As the female comes to the age of oviposition, the ova may be seen within her body in various stages of development. They appear both cephalad and caudad of the genital pore. Oviposition may be slow at first, and the first eggs can be seen to pass out and slip along under the cuticula. This is seen plainly in living females at the beginning of oviposition. The nature of this cuticular egg storage chamber may be studied in glycerin jelly mounts of such females in toto or by means of sections. The egg (Fig. 5) possesses a fairly thick shell, somewhat testaceous in color. It appears somewhat disk-shaped. The photograph shows them in flat view. When an egg is placed upon edge it forms an oblong outline, well shown in sections. It measures 33.2μ in diameter and 16.6μ in thickness. In the egg-burdened female will be found eggs in all stages of development, from unsegmented eggs to those containing actively coiling embryos, the latter not being abundant until the egg capsule is fairly well started.

NUMBER OF EGGS PER FEMALE PARASITE

Host	Size of Host	No. Parasites		Size of Parasites, Female	Total Number Eggs	Remarks
		Female	Male			
Pupa	Small	1	3	5 mm.	1,262	Embryos, all stages
Adult	Normal	1	6	5.2 mm.	2,046	Embryos, all but late coil stage
Larva	Enlarged	1	?	?	5,123	All stages of embryo
Larva	4 mm.	1	3	5 mm.	262	Egg capsule just beginning
Larva	6 mm.	1	2	4.9 mm.	1,763	Still egg laying
Larva	5 mm.	1	3	5 mm.	1,240	Still egg laying
Adult	Abdomen enlarged	1?	2	5.1 mm.	2,484	
Larva	5 mm.	1	1	5.1 mm.	5,520	
Larva	6 mm.	1	3	5 mm.	2,005	
Larva	7 mm.	1	2?	5 mm.	3,750	
Larva	7 mm.	2	5	5 mm.	4,700	

Incubation and Hatching.—Eggs containing mature embryos from young females, when dissected out and placed in water, hatch within twenty-four hours; eggs from old females hatch very shortly, from a few minutes to a few hours. The embryo may be seen coiling about within the shell for some time before it forces its way out. Newly hatched larvae are of two kinds, a very slender form with a curve at the caudal end 125μ long, and a plumper, slightly curved form 90μ long. The former, in hatching, often has some difficulty in freeing itself from the egg. In one case the little worm struggled for a half hour; it was free save for the caudal end, which hooked firmly into the

egg shell. The nema twisted about through the water, stopping now and then to give the whole body a series of vibrations in an attempt to get free. It was thirty minutes before a sudden and forceful effort set it at liberty.

Dispersal.—The distribution of this nematode may take place by migration of the maggots, through infested flies, and through the agency of air or water. The heavily infested maggot disintegrates and the nematode eggs mix with the soil to be eaten by other maggots or tossed about by wind or water. Dispersal by adult flies was proved in the laboratory. Three potted plants containing eggs and very young sciara maggots were placed in the same rearing cage with a can of earth containing infected maggots. From this can infected adult flies flew to the other pots, where some of them died. The dead bodies were full of nematode eggs which shortly brought about an infection of the maggots in the flower pots.

EXPLANATION OF PLATE

Fig. 1. Normal sciara maggot showing large white fat bodies and segmentally arranged discs of adipose tissue.

Fig. 2. Sciara maggot in advanced state of parasitism; white female nematode within.

Fig. 3. Mating female *Tetradonema plicans*; two males attached; egg laying already begun.

Fig. 4. Gravid female nematode swollen by eggs retained beneath cuticula.

Fig. 5. Eggs in various stages of development.

Fig. 6. Female nematode with reproductive organs dissected out.





THE LONGEVITY OF THE FISH TAPEWORM OF MAN, *DIBOTHRIOCEPHALUS LATUS*

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While many species of tapeworms are known to be but short-lived within their host, it is well known that under favorable conditions others may live for a considerable period. This seems especially true of *Taenia saginata*, of which Leuckart says:

"The life of the present species seems to be very long. At any rate it is not at all rare for patients to evacuate proglottides almost daily for years. One of my Russian students harbored two tapeworms for more than five years. In another case the disease continued for more than eight years. Wawruch mentions several cases which lasted from twenty to twenty-five years, and in one case speaks even of thirty-five years."

There are also on record a number of cases of persisting infestation on man by the fish tapeworm, *Dibothriocephalus latus*, some of them evidently unquestionable, others complicated by the possibility of reinfection. Two cases in the clinical records of the University Hospital, Minneapolis, Minnesota, seem especially clear cut and of such interest as to justify publication. For permission to use the data I am under obligations to Dean E. P. Lyon of the Medical School.

1. Mrs. X—, for four years a resident of Minneapolis, was admitted to the hospital July 12, where on August 24, she gave birth to a child. On August 31 it was noted that her stools contained segments of *D. latus* and ova of the same. September 1, after appropriate treatment, she evacuated a complete specimen, with head, of *D. latus*.

The patient was a Russian Jew who had left Russia some five years previously. She had been "troubled a good deal with gas on the bowels and cramps during the past five years."

2. Mrs. Y—, a Swedish woman, 43 years of age, was admitted to the University Hospital October 13, 1911, with a complication of diseases. On account of a high degree of eosinophilia which the patient maintained while in the hospital the possibility of parasitic infestation was suspected, and on examination of the stools many eggs of *D. latus* were found. Treatment was instituted and a worm recovered complete, after which the eosinophilia dropped from 25 to 2.8 per cent.

The records show that tapeworm segments were again found in the stools on February 18, 1912, and March 4, but no heads were found. The patient was discharged, but was readmitted July 3, 1912, with diagnosis of "*B. latus*, Addison's disease, tuberculosis of the lymph glands." After treatment, 15 cm. of the worm, but no head, were discharged. Eggs and segments continued to be noted in stools up to August 21, when, following treatment, some 20 m. of worms and two heads were discharged.

The patient stated that she had had worms since fifteen years of age. From the time when she first began to menstruate at the age of 14, she often noticed worm segments, and sometimes was able to extract long pieces of worm. The segments were of a very white color. One day she told her mother about it, but as they never bothered her, she neither consulted a doctor nor at any time took any medicine to expel the worm.

She was never ill until she was 29 years of age. It was at this time that she came to the United States from Sweden, and while aboard ship she became very sick. She vomited severely, the vomitus containing pieces of the tapeworm. The worms from which these segments were derived had not been expelled previous to the patient's entering the hospital.

Since coming to the United States she had lived for two years in Brooklyn, three years in Michigan, three years in Wisconsin and five years in Minnesota.

Though it is clear that the first infestation in this case occurred at least twenty-nine years previously, this, of course, does not prove that the age of the worms expelled was that great. Under appropriate conditions of environment and food habits, repeated infections may have occurred during the early life of the patient. The age of the worms recovered at the hospital was at least the thirteen years covered by the period of residence in this country—how much greater, it would be impossible to judge.

Concerning both of the cases here reported, it may be objected that there is evidence that *Dibothriocephalus latus* is endemic in some sections of this country. That this does not account for these cases seems evident from the extreme rarity of native infestation, the fact that both patients had lived in the large cities, rather than in the region of the suspected lakes, and especially from the clear history of infestation before coming to this country.

WINTHROP DAVENPORT FOSTER

Winthrop D. Foster died in Washington, D. C., on October 6, 1918, as a result of pneumonia complications following an attack of influenza. He is survived by his wife, formerly Miss Christian Kershaw of Windsor, Ontario, and three children. Mr. Foster was born in Jersey City, N. J., December 28, 1880, and was the son of a Congregational clergyman, Dr. Addison P. Foster. The family was from New England, and Mr. Foster attended the Roxbury Latin and Newton High Schools, graduating from the latter in 1900. He later attended Williams College, from which he received the degree of B.A. in 1904, and the degree of M.A. in 1912.

He also studied forestry at the University of Michigan and veterinary medicine at George Washington University. In 1904-05 he was instructor in biology in Assumption High School at Assumption, Illinois, and from 1908 to 1910 was clerk-translator in the Census Bureau. In 1910 Mr. Foster became Junior Zoologist in the Zoological Division of the Bureau of Animal Industry, and was connected with that division up to the time of his death. He was one of the first members of the Helminthological Society of Washington and was also a member of the Biological Society of Washington.

Mr. Foster's work in parasitology was done principally on the parasites of swine and along the lines of critical tests of the efficacy of anthelmintics, though he published a number of notes on other lines.

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NEW HUMAN PARASITE

Enteromonas bengalensis Chatterjee, 1919. This flagellate was found in India in the stool of a ten year old boy who died after suffering a year from chronic dysentery. It differs from *Enteromonas hominis* Da Fonseca, 1915, in that the nucleus does not show a differentiated karyosome; also there is no blepharoplast attached to the nucleus by a rhizostyle. (Indian Jour. Med. Research, 6: 380-382, text fig., Jan., 1919.)

NOTES

William Walter Cort (A.B., Colorado College, 1909; Ph.D., University of Illinois, 1914), who is at present on the staff of the University of California, and consulting helminthologist of the California State Board of Health, has been appointed Associate in Helminthology in the School of Hygiene and Public Health, Johns Hopkins University. His work in Baltimore will begin in the fall.

Ernest Carroll Faust (A.B., Oberlin College, 1912; Ph.D., University of Illinois, 1917), now instructor in Zoology at the University of Illinois, has accepted a position with the China Medical Board, Rockefeller Foundation, as Associate in Parasitology, Department of Pathology, Union Medical School, Peking, China. He plans to assume his duties in Peking early in October.

The Instituto Bacteriologico of Buenos Aires has been reorganized. Among the departments and appointments listed are Parasitology, Doctor Wolffhügel; Medical Zoology, Doctor Bachmann. The latter has been designated for a mission abroad.

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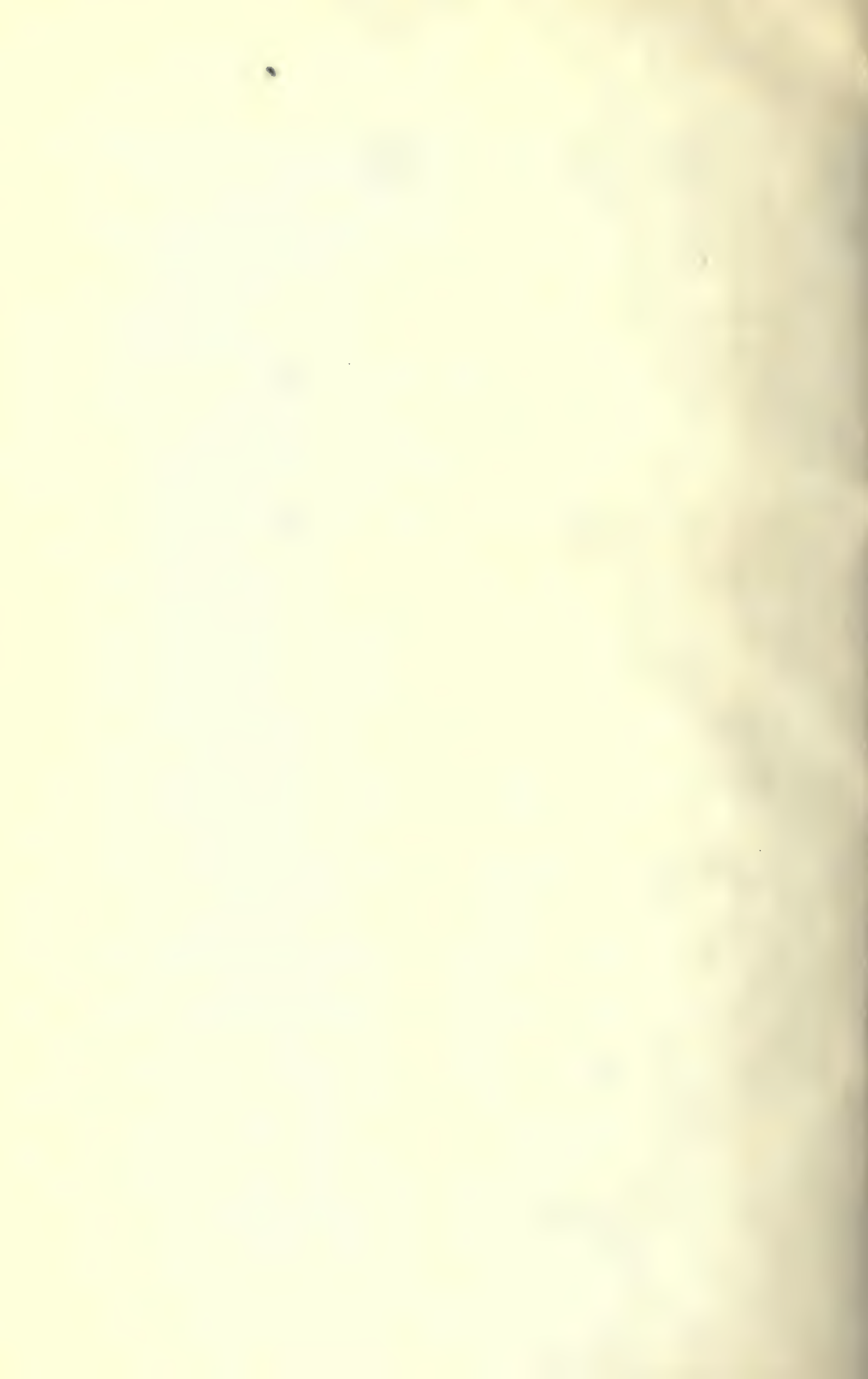
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